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African Journal of Plant Science

Full Length Research Paper

Genetic variability, heritability and variance components of some yield and yield related traits in second backcross population (BC_2) of cassava

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Second backcross population developed from crosses between wild relative *Manihot walkarae* and *Manihot esculenta* were evaluated using randomized complete block design at National Root Crops Research Institute (NRCRI) Umudike in 2011 and 2012 cropping seasons. The objective of this study was to assess the extent of genetic variability, heritability, variance components of some yield, yield related traits, pest and diseases from BC_2 population. Combined analysis of variance showed significant differences among the genotypes for all the traits evaluated. The phenotypic coefficient of variation (PCV) in the BC_2 population was higher than the corresponding genotypic coefficient of variation (GCV) for all the traits mearsured indicating the effect of environment on the expression of these traits. High heritability was recorded in some of the traits evaluated. This will aid the phenotypic selection for these traits in cassava.

Key words: BC_2 population, cassava genotypes, heritability, genetic variability, phenotyic coefficient of variation, genotypic coefficient of variation.

INTRODUCTION

Cassava (*Manihot esculenta* Crantz) is a food security crop for most of the populations in the tropical regions of the world (Akinwale et al., 2010). Cassava has moved from being a subsistence crop to a commercial crop, due to its income generating capacity and enormous potentials for industry, animal feed and human consumption.

Cassava breeders are increasingly interested in the genes of wild relatives because they offer many opportunities for transferring useful genes (Nassar, 2003)

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into cultivated germplasm. They create a great opportunity for plant breeders with variation that can be used to develop cultivars with valuable traits such as resistance to pests and diseases, high dry matter content, high storage root yield and tolerance to post-harvest physiological deterioration. The suceptibilty of Latin American germpalsm to the cassava mosaic disease has made it inevitable to explore source of CMD resistance to improve CIAT breeding populations targeted for Africa (Okogbenin et al., 2007).

Yield improvement, a major thrust in crop improvement programmes, can be achieved by exploiting the genetic variability within a gene pool. Recent studies on the genetic variability among cassava genotypes showed remarkable intra-varietal variation (Asante and Offei, 2003; Zaldivar et al., 2004). This study was conducted to evaluate the extent of genetic variability, heritability and genetic advance among genotypes in a BC2 population of cassava.

MATERIALS AND METHODS

Plant materials

The genotypes used for this study are second backcross population BC_2 generated from crosses between wild relative *Manihot Walkarae* and *M. esculenta*. *M. walkerae* was the donor parent long identified for some desirable traits of economic importance. *M. esculenta* was used as the genetic background for key desirable breeding traits such as cassava mosaic disease (CMD) resistance, good yield and dry mater content, high harvest index etc. Top elite cassava varieties from the breeding gene pool of CIAT were used as *M. esculenta* (cultivated cassava and recurrent genome).

To initiate the development of the backcross population, an interspecific cross was made between *M. walkerae* (Wae 001) and elite cassava genotype (SM909-25) to generate F_1 progenies. The F_1 progenies were backcrossed with SM909-25 and MTA18 to develop three families of first back cross population. The first backcross population BC1 were further backcrossed with cassava mosaic (CMD) resistant parental lines to develop the BC_2 population of twenty four families. The CMD parent genotypes used for the population development were either of horizontal or vertical resistance. Moderately CMD resistant check varieties (NR 8082 and TMS 30572) and susceptible check varieties (TMS 30555 and NR8212) were planted in the field to assess disease pressure on the field. Four hundred and sixty nine genotypes from three families of the BC_2 population (BC280, BC284 and BC289) were multiplied, hardened and taken to the field.

Experimental field and design

The experiment was carried out at National Root Crops Research Institute (NRCRI), Umudike in two cropping seasons (2010/2011 and 2011/2012). The experimental design used was randomize complete block design (RCBD) with single row plots of five plants each per genotype, and replicated three times. The experiment was carried out under rain fed conditions using healthy stakes (25 cm long) planted at a spacing of 1×1 m. Weeding was done manually when necessary. The research field is on latitude 05° 29[1]N, longitude 07° 24[1]N, altitude 120 m with annual rainfall of 2200 mm, temperature 26°C, relative humidity 50 to 95%, and the soil classification is dystric fluvisols.

Traits measured

Total plant height (cm) was measured from the soil level to the apical point of the plant at harvest time; the three innermost plants were used for the phenotypic assessment. Plant vigour by visual rating was scored based on the scale of 1 to 5 with score 1 as highly stunted; score 2 as stunted with very thin stems, score 3 as intermediate vigour, score 4 as vigourous; and 5 as extra vigourous. Plant architecture by visual rating was scored based on the score of 1 to 5 with score 1, good plant height (1.5m and above) and late branching; score 2 as good architecture, erect with little or no branching; score 3 as intermediate branching; score 4 as high number of branches, and score 5 as profusely branched. Root colour by visual rating was scored based on the rating of 1 to 3 where 1 is white, 2 is cream and 3 is yellow. Flowering ability was scored based on the scale of 0 to 1. 0 is no flowering while 1 is flowering. Genotypes were evaluated for resistance to cassava mosaic disease (CMD) and cassava bacterial blight (CBB) at 3, 6 and 9 months after planting. Cassava green mite was evaluated during the dry spell of the growing season. Each genotype was scored in each season, using the highest (maximum) score observed as a measure of the plant's reaction (resistance or susceptibility) to the disease. The rating of CMD is based on a severity index that consisted of five classes of symptoms: score 1 = no symptoms; score 2 = mild distortion at the base of the leaflets with the rest of the leaflets appearing green and healthy, 3 = moderately resistant with strong mosaic pattern on entire leaf, narrowing and distortion of lower than 30% of the leaflets; 4 = susceptible with severe mosaic, distortion of 60% of leaflets; and 5 = severe mosaic, distortion of more than 80% leaflets. Severity of cassava bacterial blight was also rated on a scale of 1 to 5. Where 1= highly resistant with no symptom; 2 = resistant with angular leaf spotting; 3 = moderately resistant with exclusive leaf blight, leaf wilting and defoliation, gum exudation on stems and petiole; 4 = susceptible with extensive leaf blight, wilt, defoliation and stem die back; 5 = highly susceptible with complete defoliation and stem die back. The severity index for cassava green mite (CGM) was: 1 = no symptom; 2 = scattered chlorotic on young leaves with no reduction in leaf size; 3 = severe chlorotic symptoms with slight reduction in leaf size; 4 = severe chorotic symptoms with reduced leaf size of young shoots; 5 = very severe chlorosis with significant reduction in leaf size of extensive defoliation and candle stick appearance. Cassava anthracnose was evaluated at 6 and 9 months after planting. The severity index for cassava anthracnose was: 1 = no symptoms observed; 2 = few shallow cankers on woody stems; 3 = many deep cankers on woody stems; 4 = many oval lesions on green stems; 5 = many lesions on green stems and severe necrosis of axils. At harvest, roots produced by each plant, as well as the above ground biomass (stem and foliage), were weighed. The three central plant stands per plot were harvested and used to estimate fresh root yield (tons per hac). Harvest index (HI) was measured as a ratio of weight of the harvestable roots to total biomass (root and above ground biomass). Percentage dry matter content of the roots was estimated using the specific gravity methodology. Approximately 3 kg of storage roots were weighed in a hanging scale (W_A). The same sample was weighed with roots sub-merged in water (W_W). Dry matter content was estimated as: %DMC = [158.3 × (W_A/(W_A-W_W) - 142], where W_A = weight in air and W_W = weight in water (Jaramillo et al., 2005). Dry root yield (DRY) was derived by multiplying FRY with percentage DMC.

Statistical analysis

All data generated were analyzed using SAS/STAT statistical software version 9.3 (2011). Analysis of variance (ANOVA) was carried out on a plot mean basis using the Generalized Linear Model (GLM) procedures for randomized complete block design. Estimates of variance components were obtained by equating the

Table 1. Combined analysis of variance of agronomic traits, pests and diseases resistance among progenies in BC_2 population.

Traits	Genotype mean square	Error mean square	Grand mean	Variance ratio	Min	Max
DMC[a]	29.69	15.49	26.62	1.92**	22.15	33.97
HI[b]	0.05	0.01	0.57	3.170**	0.26	0.73
FRY[c] (t/ha)	155	115.45	16.81	1.32ns	6.5	37.94
DRY[d] (t/ha)	23.2	19.68	6.37	1.18ns	2.38	17.35
PLTARCH[e] (1-5)	1.04	0.67	3.22	1.55*	2	5
PLTVIG[f] (1-5)	1.64	0.57	2.65	2.91***	1	4
PLTHT[g] (cm)	3608	1984	145.09	1.88**	77.50	245.00
PULPCOL[h] (1-3)	0.44	0.27	1.37	1.67**	1.00	2.50
FLW[i]	0.36	0.08	0.2	4.63***	0	1
Cmds[j]	3.78	0.59	1.96	6.42***	0.96	3.87
Cbbs[k]	1.06	0.54	2.68	1.97**	2.17	3.11
Cgms[l]	0.86	0.29	2.97	2.99***	2.25	4.25
Cads[m]	0.56	0.3	1.58	1.89**	1.38	2.25

[a]Dry matter content; [b]Harvest index; [c]Fresh root yield; [d]Dry root yield; [e] Plant architecture; [f] Plant vigour; [g] Plant height; [h]Pulp colour; [i]Flowering; [j]Cassava mosaic disease severity; [k]Cassava bacterial blight disease severity; [l]Cassava green mite severity; [m]Cassava anthracnose disease severity. *, **, *** significant at 5, 1 and 0.1%, respectively.

observed mean squares from ANOVA with their expected Mean squares (EMS). Phenotypic and genotypic coefficients of variation were computed using excel package.

Genotypic variance component

$$\sigma^2 g = MSg - {}^{MSe}/_{r}$$

Where *MSg* is genotypic mean square, MSe is error mean square and r is replication.

Environmental variance component

$$\sigma^2 e = {}^{MSe}/_{r}$$

Phenotypic variance component

$$\sigma_p^2 = \sigma_g^2 + \sigma^2 e$$

Genotypic and phenotypic coefficients of variation were calculated according to the method suggested by Singh and Chaudhary (1985) as:

Genotypic coefficient of variation (GCV):

$$GCV = \frac{\sqrt{\sigma_g^2}}{\bar{x}} \times 100.$$

Phenotypic coefficient of variation (PCV):

$$PCV = \frac{\sqrt{\sigma_p^2}}{\bar{x}} \times 100$$

Where $\bar{x}$ is the grand mean value of the trait
Broad sense heritability (h^2):

$$h^2 = \frac{\sigma_g^2}{\sigma_p^2} \times 100$$

RESULTS AND DISCUSSION

Trait mean and range

For each of the traits evaluated, simple statistics including the minimum, maximum, grand mean values and analysis of variance of the genotypes are summarized in Table 1. In general, the BC_2 population showed a wide range of variability for most of the characters as all the traits exhibited broad spectrum of ranges between the maximum and the minimum genotype mean values. For example, plant height ranged from 77.50 to 245.00 cm with a mean of 145 cm. Similarly, fresh root yield (FRY) ranged from 6.50 to 37.94 t/ha. Thus, it is possible to succeed in improving root yield by direct selection. Dry matter content ranged from 22.15 to 33.97% with average of 26.62% while harvest index (HI) ranged from 0.26 to 0.73. The genotypes exhibited significant variability for most traits assessed over years. These traits assessed included dry matter content, harvest index, plant architecture, plant vigour, root pulp colour, and flowering ability. Other traits include severity of diseases and pest such as cassava mosaic disease (CMD), cassava bacterial blight (CBB), cassava anthracnose disease (CAD) and cassava green mite. CMD disease pressure was high at the experimental site as the check varieties performed true to type for their disease response. A high proportion of the BC_2 genotypes were highly susceptible to cassava mosaic disease (CMD). After hardening, over 400 genotypes were successfully established on the field, but only 50 genotypes showed very good response in disease resistance to the CMD. Latin American genotypes are generally susceptible to CMD. Although parental lines having CMD resistance were used as parents in the population development, segregation in the

Table 2. Phenotypic, genotypic and environmental variances among genotypes in BC_2 population.

Traits	VP[a]	VG[b]	VE[c]
DMC	9.89	4.73	5.16
HI	0.016	0.013	0.003
FRY	51.58	13.10	38.48
DRY	7.73	1.17	6.56
ARCH	0.34	0.12	0.22
PLTVIG	0.55	0.36	0.19
PLTHT	1202.66	541.33	661.33
PULPCOL	0.15	0.06	0.09

[a] = Phenotypic variance; [b] = genotypic variance; [c] = environmental variance.

Table 3. Heritability variance estimates for yield and yield related traits in BC_2 population.

Traits	PCV[a] %	GCV[b] %	H_2b^c%
DMC	11.81	8.17	47.83
HI	22.00	20.00	81.25
FRY	42.72	21.00	25.39
DRY	40.21	16.98	15.14
ARCH	18.1	10.75	35.29
PLTVG	27.99	22.64	65.45
PLTHT	23.90	16.04	81.85
PULPCOL	28.27	17.88	40.00

[a] = Phenotypic coefficient of variation; [b] = genotypic coefficient of variation; [c]= H_2b heritability broad sense.

progenies were expected to result in good number of susceptible genotypes in the BC_2 population that were successfully hardened for field evaluation. The high significant variability among the genotypes should provide scope for selection of accessions to manage the breeding agenda.

Estimates of variance components

Plant height, fresh root yield and dry matter content exhibited high genotypic (VG) and phenotypic (VP) variances (Table 2). Genotypic coefficient of variation measures the variability of any character. The extent of environmental influence on any character is indicated by the magnitude of the differences between the genotypic and the phenotypic coefficient of variation (Akinwale et al., 2011). Large differences reflect high environmental influence while small differences reflect high genetic influence. Most of the traits exhibited high genotypic (GCV) and phenotypic (PCV) coefficient of variances (Table 3). Phenotypic coefficient of variation (PCV) values ranged from 11.81% for dry matter content to 42.72% fresh root yield whereas the genotypic coefficient variability (GVC) ranged from 8.16% for dry matter content to 22.64% of plant vigour. Phenotypic coefficient of variation (PCV) in general was higher than their corresponding GCV values for all the traits evaluated (Table 3). This indicated the considerable role of the environment on the expression of these traits; hence, variation among the genotypes is not only genetic in nature but influenced by the environment. However, lowest phenotypic and genotypic variance was recorded in harvest index. Deshmukh et al. (1986) stated that PCV and GCV values more that 20% are regarded as high, whereas values less than 10% are considered to be low and values between 10 and 20% are regarded to be medium. Based on this description, with the exception of dry matter content, GCV value was high and intermediate for the rest of the traits (Table 3). The high GCV values signify the possibility of improving these traits through selection.

Estimation of broad sense heritability

The degree of success in selection depends on the magnitude of veritable variations determined through heritability and also on genetic advance (Panse, 1957). Estimates of genetic variability within the progenies were quantified by the broad sense heritability estimates among other genetic parameters. The broad sense

heritability in the population ranged from 15.14% of dry root yield to 81.85% for plant height (Table 3). If heritability of a character is very high, (about 80% or more), selection for such characters could be fairly easy (Singh et al., 2001). This is because there would be a close correspondence between the genotype and the phenotype due to the relative small contribution of the environment to the phenotype. Selection for characters with low heritability (around 40% or less) may be difficult due to adverse effect of the environment on the phenotype. Bhateria et al. (2006) classified heritability estimates as high (>50%), medium 30%-50%) and low (<30%). With the exception of fresh root yield and dry root yield, most of the traits evaluated were highly influenced by non-genetic factors. The high genetic variation in the germplasm studied is a strong indication of significant genetic variability and low environmental variance showing great number of additive gene effect in the inheritance of these characters and these traits can be passed on to the progenies (Asante and Dixon, 2002). High broad sense heritability could be attributed to high genetic variability of the parents. This supports similar observations by Ceccarelli (1994) that the magnitude of heritability of a given trait is affected by the type of genetic material involved. Traits with moderate to high broad sense heritability coupled with high genetic advance can be improved upon through direct selection using phenotypic information.

Conclusion

High heritability of some traits indicated that these characters could be improved. It also indicated a preponderance of additive gene effect and could be transferred to the progeny in F_1 hybrids. The range and mean performance of the traits showed considerable amount of variability among the genotypes in the population. The significant differences recorded among the genotypes for all the traits evaluated also indicated variability among the genotypes. Traits evaluated showed good genetic variability in the Latin American BC2 cassava population introduced into Africa with some genotypes showing good attributes for some traits, for example, high harvest index, good vigour and plant height. This is very important in selection of parents for hybridization since crop improvement depends upon the magnitude of the genetic variability in base population. The wide range of variability observed in some of the traits evaluated may be as a result of diverse genetic background of the line of varieties studied and this could be useful in selection of genotypes for crosses.

Conflict of Interests

The authors have not declared any conflict of interests.

REFERENCES

Akinwale MG, Akinyele BO, Dixon AGO, Odiyi AC (2010). Genetic variability among forty-three cassava genotypes in three agro-ecological zones of Nigeria. J. Plant Breed. Crop Sci. 2(5):104-109.

Akinwale MG, Gregorio G, Nwilene F, Akinyele BO, Ogunbayo SA, Odiyi AC (2011). Heritability and correlation coefficient analysis for yield and its components in *rice (Oryza sativa L)*. Afr. J. Plant Sci. 5:207-212.

Asante IK, Dixon AGO (2002). Heritability studies in some cassava genotypes. West Afr. J. Appl. Ecol. 5:49-53.

Asante IK, Offei SK (2003). RAPD- based genetic diversity study of fifty cassava *(Manihot esculenta Crantz)* genotypes. Euphytica 131:113-119.

Bhateria S, Sood SP, Pathania A (2006).Genetic analysis of quantitative traits across environment in Linseed *(Linumu stitstissimum L)*. Euphytica 150(1):185-194.

Ceccarelli S (1994). Specific adaptation and breeding for marginal conditions. Euphytica 77:205-219.

Deshmukh SN, Basu MS, Reddy PS (1986). Genetic variability, character association and path coefficient analysis of quantitative traits in virginia bunch varieties of groundnut. Indian J. Agric. Sci. 56: 816-821.

Nassar MMA (2003). Gene flow between cassava, *Manihot esculenta Crantz*, and wild relatives. Genet. Mol. Res. 2:334-347.

Okogbenin E, Porto MCM, Egesi C, Mba C, Espinosa E, Santos LG (2007). Marker-assisted introgression of resistance to cassava mosaic disease into Latin American germplasm for the genetic improvement of cassava in Africa. Crop Sci. 47:1895-1904.

Panse VG (1957). Genetics of quantitative characters in relation to plant breeding. Indian J. Genet. Plant Breed. 17:318-328.

Singh RK, Chaudhary BD (1985). Biometrical methods in quantitative genetic analysis. Kalyani publisher, New Delhi, India.

Zaldivar ME, Rocha OJ, Aguilar G, Castro L, Castro E, Barrantes R (2004). Genetic variation of cassava (*Manihot esculenta Crantz*) cultivated by Chibchan Amerindians of Costa Rica. Econ. Bot. 58(2):204-213.

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African Journal of Plant Science

Full Length Research Paper

Effects of light conditions on the growth of commercial seaweed *Undaria pinnatifida*

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The artificial lighting conditions which promoted growth of the gametophytes and sporophytes of brown alga *Undaria pinnatifida* were examined. The seaweed was subjected to continuous or intermittent white, blue, or red light. There were notable, but not significant, differences in gametophyte and sporophyte growth between continuous and intermittent (10^4 Hz) white light conditions. Gametophyte growth was promoted most notably by white, followed by blue light. Sporophyte growth length was promoted most notably under intermittent white light, while body length and blade area were promoted notably under continuous white light. Sporophytes under blue or red light withered considerably. The results showed that white light is more beneficial for growth of both *U. pinnatifida* gametophytes and sporophytes compared with blue or red light. Male and female gametophyte grew more robustly under white light regardless of whether the pattern was intermittent or continuous light. However, the results further indicated that overall continuous white light promoted growth to a greater degree than did intermittent white light. Finally, white light promoted *U. pinnatifida* sporophyte growth to a greater degree than blue or red light.

Key words: Light wavelength, intermittent light, *Undaria pinnatifida*, seaweed culture, sporophyte growth.

INTRODUCTION

The development of light emitting diode (LED) devices in recent years has improved control of photo environments and resulted in great improvements to technologies for culturing plants and algae in artificial environments (Mori et al., 2002). For commercial plant culture, photo environments that promote the growth of target plant species while saving energy are needed (Takatsuji, 2010).

The brown alga *Undaria pinnatifida* is useful as a feed additive for fish farming and food for human, and is cultivated widely in coastal areas around East Asia (FAO, 2016). Techniques for culturing *U. pinnatifida* in the sea

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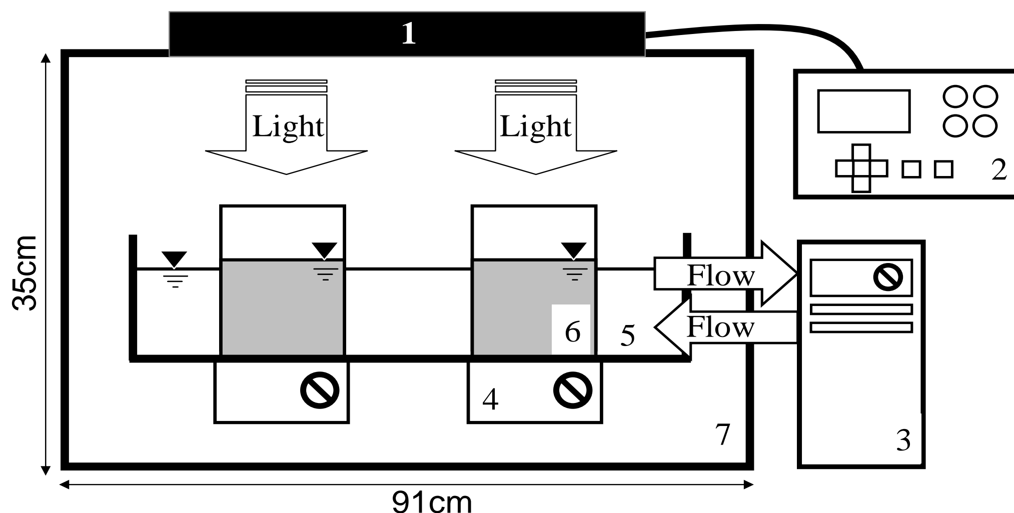

Figure 1. Experimental system. 1, LED panel; 2, LED panel control device (INS-96); 3, water temperature adjuster; 4, magnetic stirrer; 5, water bath; 6, culture vessel; 7, dark room.

are well established (Saito, 1956a; Akiyama, 1965). However, field culture production is unstable when weather conditions are poor. To ensure stable production under an artificial environment, it is necessary to identify the most effective photo environment characteristics for cultivating *U. pinnatifida*, such as the optimal light intensity, irradiance rhythms, and wavelength.

There have been some reports on the relationship between the growth of large marine plants such as *U. pinnatifida* and light intensity. It was reported that *U. pinnatifida* grows well under light intensities of 50–100 μmol $m^{-2}s^{-1}$ for gametophytes and 50 μmol $m^{-2}s^{-1}$ for sporophytes. (Akiyama, 1965; Saito, 1958; Baba, 2008; Zou et al., 2003; Morelissen et al., 2013).

Further, irradiance rhythms promote gametophyte growth and maturing sporophyte growth. Notoya et al. (1995) examined the relationship between the growth of young *U. undarioides* sporophytes and light intensity, and found that a light intensity of 80 μmol $m^{-2}s^{-1}$ and irradiance rhythm (14 h light: 10 h dark) promoted sporophyte growth. There have also been studies concerning the influence of light color on *U. pinnatifida* growth. Saito (1956b), Matsui et al. (1992), and Xu et al. (2005) examined the effects of light color on *U. pinnatifida* growth, and demonstrated that blue light is suitable for promoting the growth and maturation of gametophytes and sporophytes. However, these reports did not discuss the influence of wavelength distribution on growth in detail.

Recently, it was reported that intermittent light promotes the growth of phytoplankton (Yago et al., 2012) and lettuce (Watanabe, 1997; Yanagi et al., 1996) better than continuous light. However, no studies have examined the influence of intermittent light on the growth of macroalgae such as *U. pinnatifida*. Thus, the appropriate photo environment for *U. pinnatifida* gametophyte and sporophyte growth is unknown.

To determine a suitable photo environment for stable productive culture of the brown alga *U. pinnatifida*, the influences of intermittent light and various colors of light on gametophytes and sporophytes growing under laboratory conditions were investigated.

MATERIALS AND METHODS

Experimental apparatus

An experimental apparatus controlled the light conditions and temperature in a chamber. LED bulb panels (CCS Inc., Kyoto, Japan) were used as the light source (Figure 1). The panel was set in the constant-temperature chamber, and the lighting conditions were controlled by an external control device (INS-96; CCS Inc.). The control panel enabled adjustments to the times that lights were turned on and off, the light and dark period (below LD cycle), the frequency of intermittent lighting (10^{-2} to 10^{5} Hz) and the duty ratio (0 to 100%) of the LED panel. The LED panel included white, blue, and red lights. The spectral distributions of the LED panel lights are shown in Figure 2. The white LED had peaks at 460 and 570 nm wavelengths, while the blue and red LEDs had peaks at 470 and 660 nm wavelengths, respectively. The experimental apparatus was set in a dark room to prevent light from outside the device from entering.

Gametophyte and sporophyte

The experiments were divided two parts, according to the life stages of *U. pinnatifida*, as follows: (1) the gametophyte stage, which lasts for 14 days after substrate adhesion of the zoospore and (2) the sporophyte stage. The effects of the lighting conditions on growth were investigated during both stages.

Gametophyte stage

U. pinnatifida sporophytes cultivated off of Katsuura, Chiba, Japan, were carried to the laboratory immediately. The sporophytes were

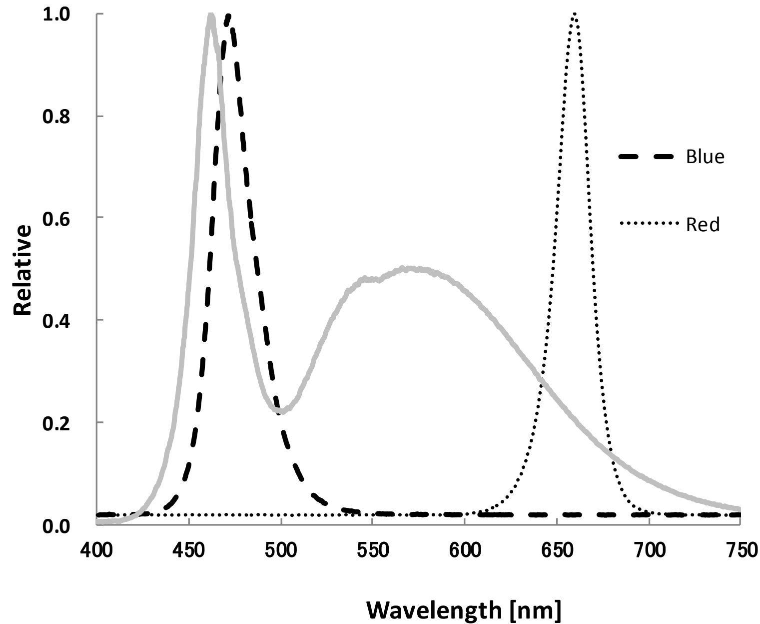

Figure 2. Distribution of the wavelengths of LEDs light sources. Solid line, dotted line, and dark dashed line indicate white, red, and blue LEDs, respectively.

dried in the shade for 1 h, and the blades of the sporangium were cut. Then, to release the zoospores, the sporangium was dipped into seawater sterilized by autoclave. The released zoospores were then poured into a 15-cm diameter, 9-cm tall petri dish that contained 1 L of sterilized seawater. Then, a glass slide was placed into the petri dish. The dish was set aside for 30 min, and the zoospores adhered to the slide glass. The adhesion density of the zoospores was ca. 400 ind./cm^2. The slide glass with the zoospores was moved to a culture vessel (Petri dish, 15 cm diameter, 9 cm height), which contained PESI culture medium (Tatewaki, 1966). The culture vessel was set in a constant-temperature room (20°C) and the zoospores were cultivated under various light conditions. The slide glass was taken out on the 7 and 14th days, and photographs of 30 individual gametophytes on the slide glass were taken using an optical microscope (100× magnification; BM-2, Olympus Co., Tokyo). Motic Image Plus 2.0S image analysis software (Motic China Group Co., Hong Kong) was used to measure the body length of gametophytes. The experiments were conducted twice for each light condition.

Sporophyte stage

Sporophytes cultivated under natural light conditions were used. The average body length of a sporophyte was 30.7±2.8 cm (n=12) at the start of the experiment. To determine the growth of the blade, a circular hole was made in the center of the blade, 3 cm from where the stipe meets the blade. Then, 2,000 mL of Provasoli's enrichment (PESI) culture medium was poured into a beaker (13 cm diameter, 20 cm height), and a sporophyte was placed in the beaker. The beakers were set in the same experimental apparatus system as the gametophytes (Figure 1). The magnetic stirrer in Figure 1 was replaced with an aeration apparatus to circulate the medium in the beaker. The temperature of the medium was 15 ± 0.5°C. The experiments were conducted three times under each lighting condition. Every 2 days, 1,000 mL of fresh PESI medium was replaced in the beaker. Sporophytes were cultured for 28 days. The body length, blade growth length, and blade area of the sporophytes were measured every 7 days. Blade growth was determined by the distance from the circular hole to 3 cm from the spot where the stipe meets the blade. A digital camera was used to photograph the sporophyte blades. Then, the blade area (including side leaves) was calculated using Adobe Photoshop CS image editing software and Lia32 image analysis software (http://www.agr.nagoyau.ac.jp/~shinkan/LIA32/LIAMan.htm).

Lighting conditions

The lighting conditions for each experiment are shown in Table 1. In the gametophyte experiments, continuous white, blue, and red light and intermittent white light were used. In the sporophyte experiments, intermittent white, blue, and red light and continuous white light were used. The intensity of the continuous light was 94 μmol $m^{-2} s^{-1}$ in the gametophyte experiment and 100 μmol $m^{-2} s^{-1}$ in the sporophyte experiment. The intensity of the intermittent light was 94 μmol $m^{-2} s^{-1}$ in the gametophyte experiment and 50 μmol $m^{-2} s^{-1}$ in the sporophyte experiment.

LD cycles were 12 h: 12 h continuous/intermittent light in the gametophyte experiments and 24 h: 0 h intermittent light (10^4 Hz) in the sporophyte experiment. Thus, the total daily quantum irradiation amounts in the gametophyte experiment and in the sporophyte experiment were equal.

RESULTS

Effects of lighting conditions on gametophyte growth

The effects of different light conditions on *U. pinnatifida* gametophyte growth are shown in Table 2. The size of the *U. pinnatifida* zoospores that adhered to the substrate slide glass was ca. 5 μm before experimental

Table 1. Experimental light conditions.

Seaweed	Irradiation light	Light color	Light intensity [μmol $m^{-2}s^{-1}$]	Frequency of intermittent [Hz]	Duty ratio [%]	L/D [hour]
Gametophyte	Continuous light	White	94	-	-	12:12
	Continuous light	Blue	94	-	-	12:12
	Continuous light	Red	94	-	-	12:12
	Intermittent light	White	94	10^4	50	12:12
Sporophyte	Continuous light	White	100	-	-	12:12
	Intermittent light	White	50	10^4	50	24: 0
	Intermittent light	Blue	50	10^4	50	24: 0
	Intermittent light	Red	50	10^4	50	24: 0

Table 2. Body length of gametophytes.

Color	Light condition	7days	14days	
		mean (SD) (μm)	male (SD) (μm)	female (SD) (μm)
White	continuous	46.8 (14.8)^b	231.0 (67.0)^a	179.6 (58.9)^a
	intermittent	56.5 (14.4)^a	220.9 (63.6)^a	179.7 (44.5)^a
Blue	continuous	41.1 (9.67)^c	173.3 (52.4)^b	137.9 (35.0)^b
Red	continuous	25.1 (7.73)^d	97.8 (33.8)^c	

Different letters within the same row indicate significant differences at p<0.05.

culture. Under continuous white light, the body length of the gametophytes was 46.8 μm on the 7th day, and those of male and female gametophytes on the 14th day were 231 μm and 179.6 μm, respectively. Under, intermittent white light (10^4 Hz), the body length of the gametophytes was 56.5 μm on the 7th day, and those of male and female gametophytes were 220.9 and 179.7 μm, respectively, on the 14th day.

The body length of gametophytes under blue light was 41.1 μm on the 7th day, and those of male and female gametophytes were 173.3 and 137.9 μm, respectively, on the 14th day. The body lengths of gametophytes under red light on the 7th and 14th days were 25.1 and 97.8 μm, respectively. Under red light, the gametophytes grew slowly; thus, we were not able to judge the sex of the gametophytes on the 14th day.

There was no significant difference in the body length of female gametophytes on the 14th day under continuous and intermittent white light ($p > 0.05$) according to a Tukey-Kramer test for multiple comparisons. However, the gametophyte length under intermittent and continuous white light was significantly greater than the corresponding lengths under blue and red light ($p < 0.01$). Thus, we found that white light effectively promotes *U. pinnatifida* gametophyte growth.

Effects of lighting conditions on sporophyte growth

Variations in body length, blade growth, and blade area of sporophytes under different light conditions over time are shown in Table 3. The measurements were conducted on the 7th, 14th, and 21st days. However, we could not take measurements on the 28th day because the tip of the blades had been destroyed, meaning that the circular holes made in the center of the blades were lost.

The body length and growth of sporophytes under continuous white light were 40.8 and 7.0 cm on the 7th day, 46.7 and 16.3 cm on the 14th day, and 50.0 and 25.0 cm on the 21st day, respectively. In contrast, body length and growth under intermittent white light were 39.2 and 9.4 cm on the 7th day, 44.3 and 18.8 cm on the 14th day, and 46.8 and 26.0 cm on the 21st day, respectively. During the experiment, the tip of the sporophyte blade deteriorated remarkably. The growth of sporophyte under intermittent light on the 7th and 14th days was 1.3 and 1.2, times greater than under continuous light. Growth on the 21st day was almost the same under continuous and intermittent light. There was a significant difference in sporophyte growth on the 7th day between continuous light and intermittent light (Scheffe's F test, $p < 0.05$). However there was no significant difference after the 14th day ($p > 0.05$). There were no significant differences in blade area under continuous and intermittent light ($p > 0.05$). Nevertheless, the blade area was larger under continuous white light than under intermittent white light.

The growth of sporophytes under blue and red light increased over time. Growth after the first week under

Table 3. Time variations of body length, growth length, and blade area of sporophyte.

Items	Color	Condition	Initial day	7th day	14th day	21th day
Body length (cm)	White	Continuous	33.7 (±0.6)	40.8 (±2.5)[a]	46.7 (±4.0)[a]	50.0 (±3.5)[a]
	White	Intermittent	28.8 (±1.0)	39.2 (±2.3)[a]	44.3 (±1.4)[a]	46.8 (±4.8)[ab]
	Blue	Intermittent	29.3 (±3.6)	38.2 (±4.3)[a]	38.2 (±3.8)[a]	37.0 (±4.4)[b]
	Red	Intermittent	30.8 (±2.8)	38.5 (±3.3)[a]	41.7 (±4.9)[a]	39.8 (±7.2)[ab]
Growth length (cm)	White	Continuous	-	7.0 (±1.3)[b]	16.3 (±3.1)[ab]	25.0 (±4.0)[ab]
	White	Intermittent	-	9.4 (±0.1)[a]	18.8 (±1.2)[a]	26.0 (±1.3)[a]
	Blue	Intermittent	-	8.7 (±0.3)[ab]	13.3 (±0.9)[b]	17.3 (±1.4)[b]
	Red	Intermittent	-	6.7 (±1.1)[b]	13.7 (±2.4)[ab]	18.8 (±4.5)[ab]
Blade area (cm^2)	White	Continuous	-	268.2 (±89.2)[a]	363.0 (±122.7)[a]	461.8 (±163.6)[a]
	White	Intermittent	-	188.2 (±31.0)[a]	232.3 (±34.3)[ab]	254.8 (±10.9)[ab]
	Blue	Intermittent	-	195.4 (±23.9)[a]	214.3 (±26.7)[ab]	137.3 (±39.1)[b]
	Red	Intermittent	-	192.2 (±44.3)[a]	169.7 (±19.6)[b]	127.2 (±32.7)[b]

Different letters within the same row indicate significant differences at p<0.05.

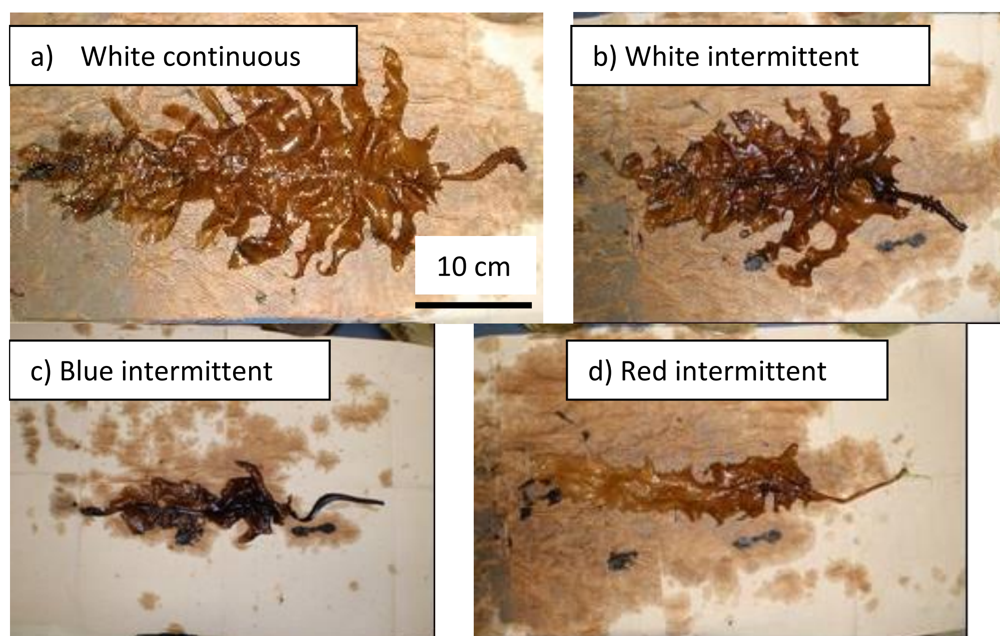

Figure 3. State of *U. pinatiffida* sporophytes at 28 days of culture. a, b, c and d display sporophytes under white continuous light, white intermittent light, blue intermittent light, and red intermittent light, respectively. The photographs were taken at the same scale.

white light was 9.4 cm, which was the greatest among the three colors of light. In contrast, growth under the red light was the least at 6.7 cm. Growth on the 21st day was 26.0, 18.8, and 17.3 cm under white, red, and blue light, respectively. Growth under white light was 1.4 to 1.5 times greater than under the other colors.

Differences in body length, blade growth, and blade area under different color lighting were examined by multiple comparison tests. There was a significant difference between body length under continuous white light and that under intermittent blue light ($p < 0.05$). There was a significant difference between blade growth under intermittent white light and that under intermittent blue light, and between blade area under continuous white light and those under intermittent red and blue light ($p < 0.01$). Body length increased most under continuous white light, while blade area increased most under continuous white light. Sporophyte blade area decreased over time under red light.

Features of sporophytes on the 28th day under each light condition are shown in Figure 3. Side leaves of the sporophytes under continuous white light grew remarkably faster than those under intermittent white light. Development of side leaves under intermittent white

light was better than that under intermittent blue and red light. Blades under blue and red light did not develop and withered.

From the above results, continuous white light promoted growth to a greater degree than did intermittent white light. Furthermore, white light promoted *U. pinnatifida* sporophyte growth more than blue or red light.

DISCUSSION

Effects of intermittent light on seaweed growth

There are many reports that the cell numbers of unicellular phytoplankton under intermittent light of various frequencies (10 Hz to 50 kHz) are greater than those under continuous light (Grobbelaar et al., 1996; Park and Lee, 2000; Janssen et al., 2001; Yoshioka et al., 2012; Yago et al., 2012). Furthermore, the same phenomenon has been reported in land plants. Watanabe (1997) reported that lettuce grew well under intermittent light conditions at a high frequency (100 Hz or more). These studies have suggested that the reason intermittent light promotes plant growth is that no light exposure is needed for the dark reaction period of photosynthesis (Park and Lee, 2000).

However, in this study, *U. pinnatifida* gametophyte and sporophyte growth were not promoted by intermittent light at a frequency of 10^4 Hz. The reason for this is not clear. In previous studies (Park and Lee, 2000), diurnal LD cycles were not used in intermittent light experiments. In the current experiment, diurnal LD cycles were used for the gametophyte experiment because a previous study showed that LD cycles affect *U. pinnatifida* growth (Notoya et al., 1995).

In this study, the light intensity of the light period in the intermittent light treatment for the gametophyte experiment was set at 188 µmol $m^{-2} s^{-1}$. Arakawa and Matsuike (1992) reported that the growth of *U. pinnatifida* gametophytes was not promoted by light intensity greater than 10.000 Lx (166 µmol $m^{-2} s^{-1}$). Thus, the light intensity we used for the intermittent lighting may have been too strong.

There were no significant differences between the growth of *U. pinnatifida* sporophytes under continuous and intermittent light. However, the development of side leaves under the intermittent light was poor (Figure 3). For sporophyte growth, the effect of having no LD cycle might be greater than the promotion of growth under intermittent light.

Effects of light color on macrophyte growth

There are many reports concerning the suitable light color environment for macrophytes (Xu et al, 2005; Matsui et al., 1992; Murase et al., 2014; Dring and Luning, 1975; Takada et al., 2011; Yago et al., 2014). The suitable light color for *E. bicyclis* (Murase et al., 2014), *Scytosiphon lomentaria* (Dring and Luning, 1975) and *Ulva prolifera* (Takada et al., 2011) growth has been examined using LEDs. These studies suggested that *E. bicyclis* and *S. lomentaria* gametophytes grew well under blue LED light and *U. prolifera* grew well under blue and red LED light. It has been suggested that the differing effects on growth by different light colors originate from the varying spectral absorptions of different species' blades.

The appropriate wavelengths for promoting *U. pinnatifida* gametophyte and sporophyte growth were investigated. It was found that both gametophytes and sporophytes grew well under white light and poorly under blue and red light.

Matsui et al. (1992) examined the effects of light color on gametophyte and sporophyte growth in several species of Laminariales. They reported that sporophytes cultured under green light and red light had an abnormal shape, while those grown under blue light grew normally. These results disagree with our current findings; however, a fluorescent lamp was used in Matsui's studies. The wavelength range of blue fluorescent light is wider than that of blue LED light (dominant wavelength; 470 nm), which may have influenced sporophyte growth. Furthermore, in contrast to blue light, the spectrum of white LED light has peaks near 500 nm and 550–650 nm. We propose that white LED light promoted *U. pinnatifida* growth because the spectral distribution of white LED light is near the photosynthetically active spectrum for *U. pinnatifida*.

The quantity of light absorbed in the blade can be determined by multiplying the photosynthetically active spectrum of *U. pinnatifida* sporophyte blades (Calogero et al., 2014) and the spectrum of each color of LEDs used in our experiment. According to these calculations, the light absorption of *U. pinnatifida* with white LED light should equal 1, and the light absorption ratios with blue and red LEDs should equal 1.3 and 0.25, respectively. Thus, the light of the blue LEDs is advantageous for photosynthesis in terms of the light absorption of the blade. However, white light promoted growth to a greater degree than blue light in our study, and the shape of sporophyte blades under white light was closer to the natural appearance of *U. pinnatifida*. Exposure to blue and red light resulted in much smaller sporophyte blades in the current study. White LED light includes a very strong blue band, a strong green band, and a weak red band. In contrast, the photosynthetically active spectrum of *U. pinnatifida* blades (Calogero et al., 2014) has two peaks at the blue and red bands. *U. pinnatifida* blades do not absorb the green light band.

In this study, an experiment using blue and red light at the same time was not conducted. The energy ratio of the blue band (400-500 nm) and red band (600-700 nm) in

white light was calculated according to their respective strengths and found that the ratio was 35:29. Thus, it was suggested that blue light and red light of the almost same strength are necessary for promoting healthy *U. pinnatifida* sporophyte growth.

Watanabe (1997) reported that lettuce exposed to 8% blue light and red light grew well and had the same shape and color as that cultivated under natural light. Mizuta et al. (2007) also investigated the effects of light color on the growth and shape of *Laminaria japonica*. They concluded that blue light assists with the formation of the sporangium, while red light restrains sporangium formation and promotes formation of the rhizoid and leaf stalk. They suggested that the use of both red and blue wavelengths is important for cultivation of large seaweed species.

In the current study, it was found that continuous white light was the most suitable artificial photo environment for *U. pinnatifida* culture. That is, the light of both blue and red wavelength bands is necessary to grow healthy *U. pinnatifida*. However, the white light also had a lower absorption by the blade than the other two colors according to the active photosynthetic spectrum of *U. pinnatifida*. To clarify the ideal photo environment for efficient *U. pinnatifida* growth, future studies should examine the appropriate ratios of blue and red light intensities for optimal growth.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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REFERENCES

Akiyama K (1965). Studies of ecology and culture of *Undaria pinnatifida* Ⅱ. Environmental factors affecting the growth and maturation of gametophyte. Bull. Tohoku Nat. Fish. Res. Inst. 25:143-170.

Arakawa H, Matsuike K (1992). Influence on insertion of zoospores, germination, survival, and maturation of gametophytes of brown algae exerted by sediments. Nippon Suisan Gakkaishi. 58:619-625.

Baba M (2008). Effects of temperature, irradiance, and salinity on the growth of *Undaria pinnatifida* from Niigata Prefecture, Central Japan. Rep. Mar. Ecol. Res. Inst. 11:7-15.

Calogero G, Citro I, Marco GD, Minicante SA, Morabito M, Genovese G (2014). Brown seaweed pigment as a dye source for photoelectrochemical solar cells. Spectrochim. Acta A Mol. Biomol. Spectrosc. 117:702-706.

Dring MJ, Luning K (1975).Induction of two-dimensional growth and hair formation by blue light in the brown alga *Scytosiphon lomentaria.* Z. Pflanzenphysiol. 75:107-117.

FAO (2016). The state of world fisheries and aquaculture 2016, Contributing to food security and nutrition for all. Rome. 200 p.

Grobbelaar JU, Nedbal L, Tichý V (1996). Influence of high frequency light/dark fluctuations on photosynthetic characteristics of microalgae photoacclimated to different light intensities and implications for mass algal cultivation. J. Appl. Phycol. 8:335-343.

Janssen M, Slenders P, Tramper J, Mur LR, Wijffels RH (2001). Photosynthetic efficiency of *Dunaliella tertiolecta* under short light/dark cycles. Enzyme Microb. Technol. 29:298-305.

Matsui T, Ohgai M, Ohshima Y, Kohara K (1992). The effects of light quality and quantity on gametophyte growth and fertility, and young sporophyte growth, in several species of Laminariales. Nippon Suisan Gakkaishi 58:1257-1265.

Mizuta H, Kai T, Tabuchi K, Yasui H (2007). Effects of light quality on the reproduction and morphology of sporophytes of *Laminaria japonica* (Phaeophyceae). Aquact. Res. 38:1323-1329.

Morelissen B, Dudley BD, Geange SW, Phillips NE (2013). Gametophyte reproduction and development of Undaria pinnatifida under varied nutrient and irradiance conditions. J. Exp. Mar. Biol. Ecol. 448:197-206.

Mori Y, Takatsuji M, Yasuoka T (2002). Effects of pulsed white LED light on the growth of Lettuce. J. Soc. High Technol. Agric. 14:136-140.

Murase N, Abe M, Noda M, Suda Y (2014). Growth and maturation of gametophyte in *Eisenia bicyclis* under different light quality from Light Emitting Diodes (LEDs). J. Nat. Fish. Univ. 62:147-152.

Notoya M, Kimura H, Ogura H (1995). Life cycle and morphogenesis of *Undaria pinnatifida* in room culture. Kaiyo Monthly 27:47-52.

Park KH, Lee CG (2000). Optimization of algal photobioreactors using flashing lights. Biotechnol. Bioprocess Eng. 5: 186-190.

Saito Y (1956a). An ecological study of *Undaria pinnatifida* Sur.-1. On the influence of environmental factors upon the development of gametophytes. Bull. Jpn. Soc. Sci. Fish. 22: 229-239.

Saito Y (1956b). An ecological study of Undaria pinnatifida Sur.-Ⅱ. On the influence of the environmental factors upon the maturity of gametophytes and early development of sporophytes. Bull. Jpn. Soc. Sci. Fish. 22:235-239.

Saito Y (1958). An ecological study of *Undaria pinnatifida* Sur.-3. On the effects of light intensity and water temperature upon the rate of photosynthesis -1. Bull. Jpn. Soc. Sci. Fish. 24:484-486.

Takada J, Murase N, Abe M, Noda M, Suda Y (2011). Growth and photosynthesis of *Ulva prolifera* under different light quality from Light Emitting Diodes (LEDs). Aquac. Sci. 59:101-107.

Takatsuji M (2010). Present status of completely-controlled plant factories. J. Soc. High Technol. Agric. 22:2-7.

Tatewaki M (1966). Formation of a crustaceous sporophyte with unilocular sporangia in *Scytosiphonlomentaria*. Phycologia 6:62-66.

Watanabe H (1997). Light emitting diodes as the irradiation source for plant factories. Rev. Laser Eng. 25:836-840.

Xu Z, Dapeng L, Hanhua H, Tianwei T (2005). Growth promotion of vegetative gametophytes of *Undaria pinnatifida* by blue light. Biotechnol. Lett. 27:1467-1475.

Yago T, Arakawa H, Fukui K, Okubo B, Akima K, Takeichi S, Okumura Y, Morinaga T (2012). Optimum Growth Conditions of Intermittent Light Irradiation for the Production of Microalga *Isochrysis galbana*. Afr. J. Microbiol. Res. 6:5896-5899.

Yago Y, Arakawa H, Akima K, Okumura Y, Morinaga T (2014). Effects of flashing light-emitting diode (LED) of several colors on the growth of the *microalga Isochrysis galbana*. Afr. J. Microbiol. Res. 8:3815-3820.

Yanagi T, Okamoto K, Takita S (1996). Effects of blue, red, and blue/red lights of two different PPF levels on growth and morphogenesis of lettuce plants. In. International Symposium on Plant Production in Closed Ecosystems. 440:117-122.

Yoshioka M, Yago T, Yoshie-Stark Y, Arakawa H, Morinaga T (2012). Effect of high frequency of intermittent light on the growth and fatty acid profile of *Isochrysis galbana*. Aquaculture 338-341:111-117.

Zou N, Zhou B, Li B, Sun D, Zeng C (2003). Effects of cell density, light intensity and mixing on *Undaria pinnatifida* gametophyte activity in a photobioreactor. Biomol. Eng. 20:281-284.

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Full Length Research Paper

Growth, symbiotic and yield response of N-fertilized and *Rhizobium* inoculated common bean (*Phaseolus vulgaris* L.)

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Common bean (*Phaseolus vulgaris* L.) is one of the most important and widely cultivated pulse crops in most developing countries. However, its cultivation is globally constrained mainly by low soil fertility and lack of improved agronomic practices. A field experiment was conducted at Hawassa University College of Agriculture, Hawassa Southern Ethiopia to determine the effect of N fertilization and *Rhizobium* phaseoli strain HB-429 inoculation on growth, nodulation, yield and yield components of common bean variety Hawassa Dume. The experiment was laid out as a randomized complete block design with three replications. Results showed significant increase in growth, nodulation, yield and yield components in plants inoculated with *Rhizobium* strain HB-429 over the control. Thus, from the results of this study, it can be concluded that *Rhizobium* inoculation with strain HB-429 is the best performing treatment to be recommended for profitable grain yield of common bean at the Hawassa and other similar areas.

Key words: Common bean, grain yield, nitrogen, nodulation, *Rhizobium* inoculation.

INTRODUCTION

Common bean (*Phaseolus vulgaris* L.) is a major grain legume grown and consumed in sub Saharan Africa, including Ethiopia. It is the most important food legume, fodder and cover crop (Gidago et al., 2012). It matures early, has wider ecological adaptation and broad range of local genetic diversity (Fikru, 2007). Nutritionally, common bean grains are rich in protein, carbohydrates, oil, fiber and sucrose (Gebre-Egziabher et al., 2014; Tekle et al., 2014). Its green leaves and young pods also contain high levels of essential nutrients (USAID, 2012; Rocha-Guzman et al., 2007). Thus, inclusion of common bean in the daily diet has several health benefits such as reduction of cholesterol level (Rosa et al., 1998), reduction of coronary heart diseases, favourable effects against cancer (Oomah et al., 2005), decreasing diabetes and obesity and high antioxidant capacity (Mitchell et al., 2009).

Common bean has a potential of 5 t/ha grain yield (Graham and Ranalli, 1997). However, in most African countries average yield of this crop is often less than 1.0

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t/ha. For example, average yield ranges were: 0.14 to 0.77 t/ha in Kenya (Kapkiyai et al., 1998), 0.6 to 0.8 t/ha in Uganda (Kalyebara, 2008), and 0.5 to 0.8 t/ha in Ethiopia (EPPA, 2004). This implies that farm yield of common bean in Africa ranges far below its potential. This situation has been attributed to low soil fertility, poor agronomic practices, and biotic and abiotic stress during the growth of the plant (Polania et al., 2016). To alleviate these constraints and exploit the genetic yield potential of common bean more effort is needed among others, assessing them under different agronomic practices. As a result the yield potential of common bean has been raised and the risk associated with the production of crop has been minimized, leading to increased grain yield per unit area, thus improving food security among small holder farmers.

Inoculation with effective *Rhizobium* strains substantially increases the nitrogen fixing potential and yields of legumes, including common bean. However, farmers have a wrong notion that common bean, being a legume crop, does not need any nutrition and usually grow it on marginal land without applying any fertilizer. This seems to be an important reason for its low seed yield in Ethiopia. This constraint could be alleviated through seed and/or soil inoculation with the proper *Rhizobium* bacteria before or at planting to facilitate N-fixation (Ndakidemi et al., 2006). Therefore, to increase the productivity of the farmers, it is crucial to increase the awareness of farmers towards the utilization of improved agronomic practices that increase their production and accelerate food security through proper implementation. To this end, a field study was initiated with the objective of assessing growth, symbiotic and yield response of N-fertilized and *Rhizobium* inoculated common bean.

MATERIALS AND METHODS

Description of experimental site

The experiment was conducted in Hawassa University, College of Agriculture which is located at 273 km south west of capital Addis Ababa in South Nation Nationalities and People Regional State. The site is located 7° 4'N latitude and 38° 31'E longitude and an altitude of 1969 m. The average rainfall of the area is 800 to 1100 mm annually. The average annual maximum, minimum and mean temperature of the area is 27, 12, and 20°C, respectively. The main rainy season extends from April to September and it is interrupted by some dry sun shine and sometimes from May to July (NMA, 2015).

Source of planting material and experimental design

Seeds of common bean (*P. vulgaris* L.) variety Hawassa Dume were obtained from Hawassa Agricultural Research Centre. This variety was purposefully chosen based on its adaptation, high grain yield, acceptability by farmers and seed availability. Seeds were planted at the beginning of May 2016 in a complete randomized block design with three treatments and three replicates. Treatments were inoculation of bean with HB-429, addition of 23 kg N/ha, and an untreated control.

The total number of plots was 9 on each plot, there were 4 rows. In each plot, seeds were planted (two seeds per hole) with 10 cm between plants and 40 cm between row spacing. A total number of plants per experiment were 360 and 40 plants on each plot. Before sowing, seeds were pre-inoculated with a peat-based *Rhizobium* inoculant strain HB-429 in the shade and inoculated seeds were allowed to air dry for few minutes before planting. This *Rhizobium phaseoli* strain HB-429 was previously proven to enhance the symbiotic performance and yield of common bean under field condition, and it is considered an elite strain for common bean cultivation in Ethiopia (Tarekegn, 2012). After sowing, seeds were covered with soil to avoid desiccation. For the N-treatment, urea (23 kg N/ha) was applied by hand to designated plots at planting and 4 weeks after sowing. Weeds within plots were removed manually two weeks after seedling emergency and three weeks later. To avoid cross contamination, weeding was done in the un-inoculated plots first. Field management like watering, cultivation, weeding and others were carried out as recommendation.

Soil sampling and analysis

Before planting, soil samples were randomly taken from the experimental field at a depth of 0 to 30 cm using an auger and the samples were mixed thoroughly to produce one representative composite sample of 1 kg. The soil sample was air-dried and ground to pass 2 and 0.5 mm (for total N) sieves and analyzed for total N, available P, pH, organic carbon (OC), exchangeable cations and physical properties at Hawassa University College of Agriculture Soil Laboratory. Soil analysis was made as per the standard laboratory procedure (Sahlemedhin and Taye, 2000). The soil pH was measured in the supernatant suspension of a 1: 2.5 soil to water ratio using a standard glass electrode pH meter (Rhoades, 1982). The Walkley and Black (1934) method was used to determine the organic carbon (%). Total N was determined using Kjledahl method as described by Bremner and Mulvaney (1982). Available P (mg/kg) was determined by Olsen et al. (1954) method using ascorbic acid as the reducing agent. The cation exchange capacity (CEC) in cmol (+) kg^{-1} was measured using 1 M-neutral ammonium acetate method (Jackson, 1967). The soil-particle size distribution was determined using the Bouyoucos hydrometer method (Bouyoucos, 1962).

Data collection

Plant height

Five plants from the central rows of each plot were randomly selected for measuring plant height. Then the average values of these plants were recorded as plant height of the crop.

Shoot dry matter

Shoots dry matter was determined at early pod setting stage from plants that were sampled for plant height and nodulation. The plant samples were placed in labeled perforated paper bags and oven dried at 70°C for 48 h until a constant shoot dry matter.

Nodule number/plant:

Nodulation assessment was undertaken at mid (50%) flowering stage by carefully uprooting five plants randomly from each plot. The plants were separated into shoot and roots. The adhering soil was carefully washed from the roots over a metal sieve. The nodules from each plant were picked and spread on the sieve to

Table 1. Effect of N fertilization and *Rhizobium* inoculation on plant height, shoot dry weight, nodule number and nodule dry weight of common bean.

Treatments	Plant height (cm)	Shoot dry weight (g)	Nodule number	Nodule dry weight (g)
Control	37.5[b]	20.3[b]	36.6[c]	0.37[b]
Strain HB-429	41.2[a]	31.3[a]	47.6[a]	0.60[a]
23 kg N/ha	40.8[a]	26.1[a]	41.0[b]	0.46[b]
Significance level	*	*	**	*
CV (%)	2.9	9.7	4.6	11.0
LSD	2.7	5.7	4.4	0.1

Means with the same letter(s) within a column are not significantly different at $p < 0.05$.

drain water from their surface. Nodules were counted and their average was taken for plots as nodule number/plant. Then after, the nodules were oven-dried at 70°C for 48 h for nodule dry weight determination.

Yield and yield components:

At harvesting time for the determination of yield and yield components such as number of pods/plant, number of seeds/pod, hundred seed weight and grain yield, ten randomly picked plants were used. Seed weight was determined by randomly taking 100 seeds of the ten sample plants and weighing it with sensitive balance after oven drying to constant weight.

Statistical analysis

The obtained data were subjected to analysis of variance (ANOVA) using the General Linear Model (GLM) of the Statistical Analysis System (SAS, 2002) version 9.0. Mean separation was done using Least Significant Difference (LSD) test at 5% probability level. Uninoculated plants and plants grown in non-N amended soil served as control.

RESULTS AND DISCUSSION

Soil physico-chemical properties of the study area

Analysis of the top soil (0 to 30 cm) before the application of treatments revealed that the soil at the experimental site is clay-loam in texture, slightly acidic in reaction with a pH value of 6.1, which is in the optimum range for common bean production (Havlin et al., 1999). The soil has low total N (1.0 mg/g) and organic carbon content (6.1 mg/g) as per the limit set by Tekalign (1991). Extractable available P (6.90 mg/kg) was in the range of medium, and the cation exchange capacity (CEC) was 23.6 cmol/kg and could be rated as medium and satisfactory for agricultural crops with the use of agronomic practices (Landon, 1991). The exchangeable cations K 3.5 cmol/kg, Ca 5.8 cmol/kg and Mg 2.9 cmol/kg were within the range of medium to high (FAO, 2006).

Effect of N fertilization and *Rhizobium* inoculation on plant growth and nodulation

Plant height

N fertilization and *Rhizobium* inoculation had significant ($P \leq 0.05$) effect on plant height (Table 1). The shortest plant height (37.5 cm) was recorded from the control, which was significantly lower to other treatments. Both the *Rhizobium* inoculated and 23 kg N/ha applied plants performed similarly on plant height. The reasons for increase in plant height under inoculation and N fertilizer could be due to the increased vegetative growth with applied N and nitrogen fixation. This result is in line with the report of Kubota et al. (2008) who stated that plant height of soybean was increase with N in the presence of *Rhizobium* inoculants. Other authors also reported similar results from researches conducted on chickpea and faba bean, El-Wakeil and El-Sabai (2007) indicating that *Rhizobium* inoculation significantly increased plant height.

Shoot dry weight

N fertilization and *Rhizobium* inoculation had significant effect on shoot dry matter accumulation of common bean compared to the control (Table 1). Inoculation with strain HB-429 gave relatively higher shoot dry weight that was greater by 102.3% over the control. However, statistically significant variation on shoot dry weight was not detected between the strains HB-429 and 23 kg N/ha. The observed benefits on bean by *Rhizobium* inoculation seem to be to the supply of N to the crop through symbiotic N_2-fixation (Togay et al., 2008). Similar to this result, the research outcomes of Bhuiyan et al. (2008) showed that the highest dry matter accumulation on mung bean was obtained from inoculation with *Rhizobium*. Sharma et al. (2000) reported the significant effect of seed inoculation on dry weight biomass compared to the control treatments.

Table 2. Effect of N fertilization and *Rhizobium* inoculation on yield and yield components of common bean.

Treatments	Number of pods/plant	Number of seeds/pod	Hundred seed weight (g)	Grain yield (t/ha)
Control	9.7[c]	4.4[b]	31.5[b]	1.4[b]
Strain HB-429	14.2[a]	7.7[a]	42.7[a]	2.5[a]
23 kg N/ha	11.8[b]	6.7[a]	40.5[a]	2.4[a]
Significance level	**	*	**	*
CV (%)	5.9	14.2	3.8	16.7
LSD	1.6	2.0	3.3	0.8

Means with the same letter(s) within a column are not significantly different at $p < 0.05$.

Nodule number/plant

Nodule number/plant was significantly ($P<0.01$) affected by N fertilization and *Rhizobium* inoculation (Table 1). The maximum mean nodule number (47.6) was recorded from the strain HB-429 and, zero application of treatments result the minimum nodule number per plant, which was markedly lower than the effect of other treatments. The increased nodule number with *Rhizobium* inoculation could be associated with the efficiency of introduced rhizobia, to compete with indigenous bacteria dwelling in the soil. These results are in line with the findings of Sajid et al. (2011) who revealed that the *Rhizobium* inoculation significantly enhanced nodule number. Similarly, application of N fertilizer at a rate of 23 kg N/ha increased nodule number/plant when compared to the control. However, Kessel and Hartley (2000) observed a significant decrease in nodulation of several varieties of common beans following the application of 40 kg N/ha. The lower native total N (0.10 mg/g) contents observed on the surface soil of the experimental field may have positively affected crop response and increment in common bean nodule number under application of mineral N fertilizer. This is further supported by Omoregie and Okpefa (1999), who noted that when initial levels of available soil nitrogen were low, a period of nitrogen hunger can reduce nodulation.

Nodule dry weight/plant

The effects of N fertilization and *Rhizobium* inoculation on the nodule dry weight of common bean were found to be statistical significant (Table 1). Strain HB-429 had the highest nodule dry weight (0.60 g) while the control and 23 kg N/ha treatments had the lower nodule dry weight (0.37 and 0.46 g), respectively. Similar effects of seed inoculation on nodule dry weight have also been reported by Dereje (2007) and Bhuiyan et al. (2008) on soybean who stated that inoculation significantly increase nodule dry weight of legumes under field condition. According to Fatima et al. (2006) high nodule dry weight can be generally a prerequisite for increasing N_2-fixation in legumes rather than number of nodules.

Effect of N fertilization and Rhizobium inoculation on yield and yield components of common bean

Number of pod/plant

The analysis of variance revealed the number of pods/plant was significantly affected by N fertilization and *Rhizobium* inoculation (Table 2). The lowest number of pods/plant (9.7) was recorded from the control which was significantly lower to both *Rhizobium* strain HB-429 inoculated and N fertilizer applied treatments. The increased pod number with applied inoculants and N fertilizer could be associated with enhanced growth and higher assimilate accumulation which resulted from better N nourishment due to symbiotic N_2-fixation and applied N. The result is in agreement with the work of Malik et al. (2006) and Dereje (2007) who conclude that increased number of pods per plant with *Bradyrhizobium japonicum* inoculation in soybean.

Number of seeds/pod

The analysis of variance revealed that number of seeds/pod was significantly affected by N fertilization and *Rhizobium* inoculation (Table 2). The lowest number of seeds/pod (4.4) was recorded from the control which was significantly lower than both *Rhizobium* inoculation and N application. The magnitude of increase in number of seeds/pod over the control and application of 23 kg N/ha due to inoculation of strain HB-429 was 175 and 115%, respectively. The result shows that N fertilization and *Rhizobium* inoculation played an important role in common bean generative growth and therefore made a marked increase in the number of seeds/pod. The significant difference on number of seeds/pod among inoculation/N fertilizer and the control was in line with the finding of Muhammad (2002) who found that, seed inoculation increased the number of seeds/pod in addition to grain yield.

Hundred seed weight

Significant differences ($P<0.01$) were observed among N

fertilization and *Rhizobium* inoculation for hundred seed weight (Table 2). Inoculation with strain HB-429 gave relatively heavier seed weight that was greater by 136% over the control. However, statistically significant variation in hundred seed weight was not detected between strain HB-429 and 23 kg N/ha. This result is in line with the finding of Ali et al. (2004) that states inoculation brought a significant effect on hundred seed weight of chickpea.

Grain yield

As observed in all yield parameters in the experiment, grain yield was also highly affected by N fertilization and *Rhizobium* inoculation (Table 2). The highest grain yield (2.5 t/ha) was recorded from plants inoculated with *Rhizobium* strain HB-429 and it was followed by 23 kg N/ha, which did not vary significantly between each other but significantly higher than the control. The lowest grain yield (1.4 t/ha) was recorded from the control and was significantly lower than the rest of the treatments. Yield increments of 179 and 171% were obtained from strains HB-429 and 23 kg N/ha, respectively as compared to the control. The significant increase in grain yield in response to *Rhizobium* stain HB-429 inoculation might be attributed to the increased availability of N in the soil for uptake by plant roots, through fixed N_2. The results coincide with the findings of Sajid et al. (2011), who concluded that the treatments with *Rhizobium* inoculation gave higher grain yield than those without inoculation. It may also be due to more number of pods and seeds due to *Rhizobium* inoculation and applied N. A similar increasing effect of *Rhizobium* inoculation on grain yield of soybean has also been reported by Abbasi et al. (2008). On the other hand, the increases in grain yield with N application support the finding of Nebret and Nigussie (2012) who reported that common bean crop supplied with 23 kg N/ha resulted in significantly more grain yield than the control.

Conclusion

Application of N fertilizer and *Rhizobium* inoculation resulted in significant improvement on plant height, shoot dry weight, nodule number and nodule dry weight. In this study, inoculation of common bean with strain HB-429 increased nodule number and nodule dry weight by 130 and 162% as compared to the control treatment. Thus, indicating the effectiveness of this strain in N_2-fixation. Similarly, for yield and yield components N fertilizer and *Rhizobium* inoculation showed significant effects on all parameters and strain HB-429 appeared to be more productive than all other treatments, though in some parameters it was not significantly different with 23 kg N/ha. For instance, inoculation of common bean with strain HB-429 increased number of pods/plant, number of seeds/pod and hundred seed weight by 146.3, 175.0 and 135.6% over the control, respectively. The significant differences in these test parameters had contributed to the superior grain yield performance of strain HB-429 (2.5 t/ha) compared to the control (1.4 t/ha). Generally, the result of this study indicated that treatments under fertilizer and *Rhizobium* inoculation gave the highest growth and yield related parameters of common bean plant. Thus, based on the finding, using effective *Rhizobium* strain was advisable to achieve optimum common bean yield under Hawassa condition.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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REFERENCES

Abbasi MK, Majeed A, Sadiq A, Khan SR (2008). Application of *Bradyrhizobium japonicum* and phosphorus fertilization on growth, yield and nodulation of soybean in the sub-humid hilly region of Azad Jammu and Kashmir. Plant Prod. Sci. 58:368-376.

Ali A, Khan MA, Randhawa SA (2004). Interactive effect of seed inoculation and phosphorus application on growth and yield of chickpea (*Cicer arietinum* L.). Int. J. Agric. Biol. 6(1):110-112.

Bhuiyan MMH, Rahman MM, Afroze F, Sutradhar GNC, Bhuiyan MSI (2008). Effect of Phosphorus, Molybdenum and *Rhizobium* Inoculation on Growth and Nodulation of Mungbean. J. Soil Nat. 2(2):25-30.

Bouyoucos GJ (1962). Hydrometer method improved for making particle size analysis of soils. Agron. J. 54:464-465.

Bremner JM, Mulvaney CS (1982). Methods of Soil Analysis, Part 2 Chemical and Microbiological Properties, Pp. 595-624.

Dereje A (2007). Effect of *Bradyrhizobium japonicium* inoculation and N fertilizer on nodulation, protein content, yield and yield components of soybean (*Glycin max* L.) in Awassa. Msc thesis. Hawassa University. Ethiopia.

EPPA (2004). Ethiopian Export Promotion Agency. Ethiopian pulses. Product Development and Research Directorate. Addis Ababa, Ethiopia. Pp.12-15.

El-Wakeil NE, El-Sebai TN (2007). Role of Biofertilizer on Faba bean growth, yield, and its effect on bean aphid and the Associated Predators. Res. J. Agric. Biol. Sci. 3(6):800-807.

FAO (Food and Agriculture Organization) (2006). Plant nutrition for food security: A guide for integrated nutrient management. FAO, Fertilizer and Plant Nutrition Bulletin 16, Rome.

Fatima Z, Zia M, Chaudhary MF (2007). Interactive effect of *Rhizobium* strains and p on soybean yield, nitrogen fixation and soil fertility. Pak. J. Biotechnol. 39(1):255-264.

Fikru M (2007). Haricot ban (*Phaseolus vulgaris* L.) variety development in the lowland areas of Wollo. Proceedings of the 2nd Annual Regional Conference on Completed Crops Research Activities 18 - 21 September 2007, Bahir Dar, Ethiopia, pp. 86-93.

Gebre-Egziabher Murut, Hadush Tsehaye, Fetien Abay (2014). Agronomic performance of some haricot bean varieties (*Phaseolus vulgaris* L.) with and without phosphorus fertilizer under irrigated and

rain fed conditions in the Tigray and Afar regional states, northern Ethiopia. Momona Ethiop. J. Sci. 6(2)95-109.
Gidago G, Beyene S, Worku W (2012). The response of haricot bean (*Phaseolus vulgaris* L.) to phosphorus application on ultisols at Areka, Southern Ethiopia. J. Biol. Agric. Healthc. 1(3):38-49.
Graham PH, Ranalli P (1997). Common bean (*Phaseolus vulgaris* L.). Field Crops Res. 53:131-146.
Havlin JE, Beaton JD, Nelson WL, Tisdal SL (1999). Soil Fertility and Fertilizers. An Introduction to Soil Management, 6th ed. Prentice Hall, Inc. 634p.
Jackson M (1967). Soil chemical analysis. 1958. New Jersey, Prentice Hall, P. 219.
Kalyebara R (2008). The impact of improved bush bean genotypes in Uganda. Network on Bean Research in Africa, Occasional Publication Series, (43).Kampala, Uganda: CIAT.
Kapkiyai J, Karanja N, Woomer P, Qureshi J (1998). Soil organic carbon fractions in a long-term experiment and the potential for their use as a diagnostic assay in highland farming systems of central Kenya highlands. Afr. Crop Sci. J. 6(1):19-28.
Kessel CV, Hartley C (2000). Agricultural management of grain legumes: has it led to an increase in nitrogen fixation. Field Crops Res. 65:165-181.
Kubota A, Hoshiba K, Bordon J (2008). Effect of fertilizer-N application and seed coating with rhizobial inoculants on soybean yield in eastern Paraguay. Rev. Bras. Ciênc. Solo 32:1627-1633.
Landon JR (1991). Booker tropical soil manual: a handbook for soil survey and Agricultural land evaluation in the tropics and subtropics. Long man scientific and technical. Booker Tate Ltd. John Wiley and Sons, Inc., New York.
Malik A, Hassan F, Waheed A, Qadir G, Asghar R (2006). Interactive effects of irrigation and phosphorus on green gram (*Vigna radiata* L.). Pak. J. Biotechnol. 38(4):119-1126.
Mitchell DC, Lawrence FR, Hartman TJ, Curran JM (2009). Consumption of dry beans, peas, and lentils could improve diet quality in the US population. J. Am. Diet. Assoc. 109(5):909-913.
Muhammad AK (2002). Production efficiency of chickpea (*Cicer arietinum* L.) as affected by inoculation, phosphorus levels and intercropping. PhD. (Agriculture) Thesis, University of Agricultural Sciences, Faisalabad. pp. 48-79.
Ndakidemi P, Dakora FD, Nkonya E, Ringo D, Mansoor H (2006). Yield and economic benefits of common bean (*Phaseolus vulgaris*) and soybean (*Glycine max*) inoculation in northern Tanzania. Anim. Prod. Sci. 46(4):571-577.
Nebret T, Nigussie D (2012). Effect of nitrogen and sulphur application on yield components and yield of common bean (*Phaseolus vulgaris* L.) In Eastern Ethiopia. MSc. Thesis, Haramaya University, Ethiopia.
NMA -National Meteorological Agency (2015). Hawassa branch.
Olsen SR, Cole CV, Wantanabe FS, Dean LA (1954). Estimation of available phosphorous in soils by extraction with sodium bicarbonate, U.S. Department of Agriculture circular 939. U.S.D.A, Washington DC., USA. 23p.
Omoregie AU, Okpefa GO (1999). Effects of time of application of nitrogen on nodulation, dry matter and mineral nutrition of cowpea Vigna *unguiculata* L (Walp) in the Delta area of Nigeria. Niger. Agric. J. 30:32-40.
Oomah BD, Cardador-Martínez A, Loarca-Piña G (2005). Phenolics and antioxidative activities in common beans (*Phaseolus vulgaris* L). J. Sci. Food Agric. 85(6):935-942.
Polania J, Poschenrieder C, Rao I, Beebe S (2016). Estimation of phenotypic variability in symbiotic nitrogen fixation ability of common bean under drought stress using ^{15} N natural abundance in grain. Euro. J. Agron. 79:66-73.
Rhoades JD (1982). Soluble salts. In. A. L. Page et al. (ed.) Methods of soil analysis: Part 2: Chemical and microbiological properties. Monograph Number 9 (Second Edition). ASA, Madison, WI. pp. 167-179.
Rocha-Guzman NE, Gallegos-Infante JA, Gonzalez-Laredo RF, Preza AM, Lerma Y, Ibarra-Perez FJ (2007). Antioxidant Activity in Cotyledon of Black and Yellow Common Beans (*Phaseolus Vulgaris* L.). Int. J. Biol. Sci. 2(1):112-117.
Rosa C, Costa N, Leal P, Oliveira TT (1998). The cholesterol-lowering effect of black beans (*Phaseolus vulgaris* L.) without hulls in hypercholesterolemic rats. Arch. Latinoam. Nutr. 48(4):299-305.
Sahlemedhin S, Taye B (2002). Procedures for Soil and Plant analysis. National Soil Research Center, EARO. Technical paper No 74, Addis Ababa, Ethiopia.
Sajid M, Rab A, Wahid F, Shah SNM, Jan I, Khan MA, Hussain SA, Khan MA, Iqbal Z **(**2011). Influence of *Rhizobium* inoculation on growth and yield of groundnut cultivars. Sarhad J. Agric. 27(4):574-576
SAS Institute Inc. (2002). SAS User's Guide, Statistics Version 9.0 ed. SAS Institute, Cary, NC, USA.
Sharma S, Upadhyay RG, Sharma CR (2000). Effect of *Rhizobium* inoculation and nitrogen on growth, dry matter accumulation and yield of black gram (V*igna mungo*). Legume Res. 23(1):64-66.
Tarekegn YS (2012). Biological Nitrogen Fixation and Its Role in Food Security. MSc. (Agriculture). Thesis, Hawassa University College of Agricultural, Ethiopia. pp. 25-79.
Tekalign T (1991). Soil, plant, water, fertilizer, animal manure and compost analysis. Working Document No. 13. International Livestock Research Center for Africa, Addis Ababa.
Tekle Y, Getachew G, Wondewosen S, Tibebu S, Ermias M (2014). Evaluation of Common Bean [*Phaseolus vulgaris* (L.)] Varieties, for Yield and Yield Components. J. Biol. Agric. Healthc. 4(17):22-25.
Togay N, Togay Y, Cimrin KM, Turan M (2008). Effect of *Rhizobium* inoculation, sulfur and phosphorous application on yield, yield component and nutrient uptake in chick pea (*Cicer arientinum* L.). Afr. J. Biotechnol. 7(6):776-782.
USAID-US Agency for International Development (2012). Pulse CRSP-Collaborative Research Support Program.
Walkley A, Blank IA (1934). An example of the digestion method for determining soil organic matter and a proposed modification of the chronic acid titration method. Soil Sci. 34:29-38.

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Full Length Research Paper

Curvularia lunata as, a dominant seed-borne pathogen in *Dalbergia sissoo* Roxb: Its location in seed and its phytopathological effects

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Dalbergia sissoo (shisham) seeds naturally infected with _Curvularia lunata_ showed either black discoloration (1.25 to 8.75%) or appeared dull (0.50 to 10.0%). When they were incubated, they yielded pure growth of the pathogen. During location studies, cleared whole mount preparation and component plating revealed the presence of infection restricted to the seed coat and cotyledons of asymptomatic seeds. However, this pathogen penetrates the deeper tissue of symptomatic seeds to the embryonic axis. Due to heavy infection, the disintegration of cells results in the formation of lysogenic cavities. The fungus enters the seeds through the hilar region and epidermal cells of the seed coat to the aleurone layer and finally into the cotyledonary cells of the embryo. The pathogen is both externally and internally seed-borne. The internal inoculum affected seed germination, viability and caused high total (pre-and post-emergence) losses (15 to 80%). The pathogen was transmitted from seed to seedling causing heavy losses to the tree plantation.

Key words: *Dalbergia sissoo, Curvularia lunata*, pre-and post-emergence losses, phytopathological effect.

INTRODUCTION

Shisham (*Dalbergia sissoo* Roxb.), an important legume tree belonging to the family of Fabaceae and subfamily Papilionaceae, is widely grown throughout the world and in India (Ashour and El-Kadi, 1958; Champion and Seth 1968). It grows as an ornamental roadside tree (Anonymous,1952; NAS, 1983) and is used for medicinal purposes (Nadkarni, 1954). Leaf spot disease caused by *Curvularia lunata* results in severe losses to Shisham plantations (Bhowmick and Vardhan, 1981). *Curvularia* is defined as a type of species *C. lunata* (Wakker) Boedijn, which appears as shiny velvety-black, and has fluffy growth on the colony surface. It is distinguished by septate, dematiaceous hyphae producing brown, geniculate conidiophores. Its conidia are curved slightly to distinctly transversely septate with an expanded third cell from the pore end of the conidium (Nelson and

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Table 1. Seed samples of *Dalbergia sissoo* infected by *Curvularia lunata* in Jaipur.

Location	No. of samples	Dry seed examination		In standard blotter method		In PDA test
		Black	Dull	Untreated	Pretreated*	
Bani Park	3	3	3	2	2	-
Forest Training Centre	3	1	2	3	3	2
Jhalana Natural Garden	3	3	2	3	3	2
Police Academy	12	12	9	9	6	-
Sanganer	3	1	3	3	2	-
Seed Market	9	9	9	8	6	2
University Campus	12	12	12	10	9	2
VidhanSabhaBhawan	3	2	1	-	-	-
World Forestry Arboretum	3	3	3	3	2	2
Total	51	46	44	41	33	10

*Pretreated with 3% chlorine for five minutes.

Haasis, 1964). *C. lunata* is internally and externally seed-borne causing pre- and post-emergence mortality. Its pathogenicity was confirmed by artificial inoculation of healthy host tissue which resulted in disease.

Acacia nilotica has been reported during a disease survey in 2 nurseries in Jabalpur, Madhya Pradesh, India as a new host for *C. lunata* (Singh and Jammaluddin, 1995). For analysis of the host-parasite relationship (shisham and *Curvularia* sp.), artificial inoculation of the eight days old *Curvularia* sp. suspension, was sprayed on leaves of the healthy plant to prove its pathogenicity. This study revealed the occurrence of a new foliar disease with irregular leaf spots that progressed from the leaf margin to the centre of the lamina. The fungus is identified as *Curvularia* sp. while the pathogenicity test proved Koch's postulate (Sharma et al., 2012).

Four criteria were established by Robert Koch to identify the causative agent of a particular disease: the pathogen must be present in all cases of the disease, should be isolated from the diseased host and be grown in pure culture and designated as Koch's postulate or pathogenicity test to determine the host-parasite relationship (Marshall et al.,1985; Kamaluddeen and Lal, 2013). There is no information on the seed-borne nature of *C. lunata* in *D. sissoo* seeds. Therefore, the present study was carried out to know the location of the pathogen, its role in disease transmission and its phytopathological effects.

MATERIALS AND METHODS

Fifty-one seed samples collected from nine locations around Jaipur, India (Table 1) were subjected to dry seed examination and incubation tests by Standard Blotter Method (SBM) and Potato Dextrose Agar (PDA) plate test (ISTA, 1990). For the dry seed examination, shape (normal, flat, brown, reniform, asymptomatic), various discolourations (dark brown, white, black, green) and deformities (shiny, dull) were recorded. Seeds were incubated on moistened blotters with and without pretreatment with 3% chlorine for five minutes.

For PDA test, pretreated seeds were sown (10 seeds per plate) in Petri plates of 60 x 15 mm size containing PDA medium. Two seed samples were selected with high percent of incidence of the pathogen, one from the forest training centre and one from a seed market (sample accession numbers 3 and 18, respectively) with a high incidence of *Curvularia lunata*. These samples were used for location and transmission studies. The seeds are categorised as asymptomatic and symptomatic (weakly and heavily infected) based on the severity of the infection.

Location of the pathogen in different seed components was studied using component plating (10 seeds per category per sample), hand cut and microtome sectioning (five seeds per category per sample) (Singh, 2002). Transmission of seed-borne inoculum from seed to seedling/plant was studied using Petri plate method and growth tests (ISTA, 1990). For Petri plate method, four replicates of 100 pretreated seeds were sown on moistened blotters (10 seeds per plate), grown in water agar test tube seedling symptom test (1 seed/test tube), respectively. Germination, the incidence of the pathogen, seedling-symptoms, and seedling mortality were observed.

RESULTS

Out of the 51 seed samples collected during the study, they comprised either black seeds 46 (1.25 to 8.75%) or dull-appearing seeds 44 (0.50 to 10.0%) carrying infection of *C. lunata* (Table 1). Upon incubation, seeds yield pure growth of the pathogen (Figure 1A). The incidence of *C. lunata* from untreated and pretreated seeds in the SBM test was 41 (2.0 to 54.0%) and 33 (2.0 to 18.0%), respectively and 10 (10.0 to 30.0%) in the PDA test. Incidence was high in the samples from the Forest Training Center, Seed Market and University Campus (Table 1).

The sample accession was 3 and 18, subjected to histochemical localisation studies. During component plating, pathogen was detected as 15 and 25% on the seed coat, 10 and 15% on cotyledons and 0.5% on the embryonal axis of asymptomatic seeds of both samples. Characteristic mycelium with sympodial succession in conidia of *C. lunata* was observed in 50 to 80% on seed

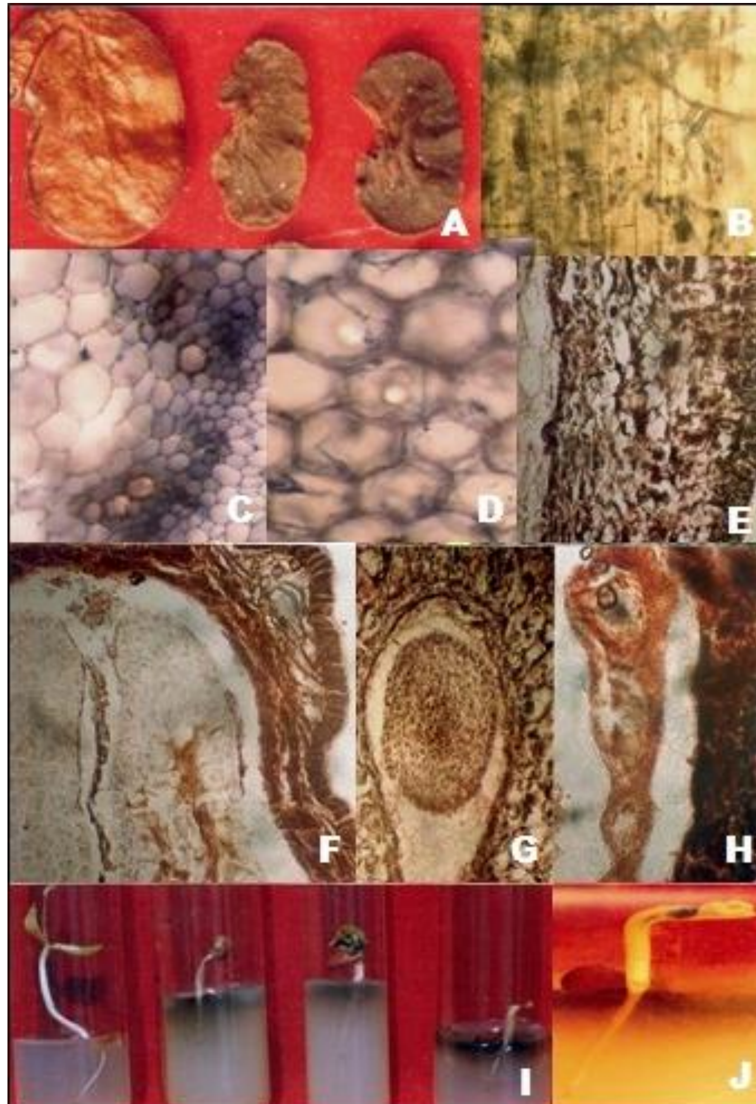

Figure 1. Histopathological and cytopathological localisation of *Curvularia lunata*in *Dalgerbia sissoo* seeds. (A)Seeds showing discolorations caused by *C. lunata* with scattered brown patches and compressed hilar region. ×18. (B) Cleared wholemount preparation of cotyledon with mycelia network. × 350. (C, D) Hand cut sections of seeds infected with *C. lunata*. Inter- and intra-cellular mycelium in cells of the cotyledons (C; ×350) and embryonic axis (D; × 700). (E-H)Microtome sections of seeds infected with *C. lunata*. (E)T.S. part of seed showing mycelial bits and cellular disaggregation in the seed coat (×700). (F)T.S. part of weakly infected seeds showing mycelial aggregation in and around the cells of the seed coat, cotyledons and the space between cotyledons (×700). (G) Inter- and intracellular mycelium in cells of seed coat and embryonal axis (×350). (H) Disintegrated cells of cotyledons of heavily infected seed (×700). (I, J) Phytopathological studies in water agar seedling symptom test showing various degrees of infection. (I) Normal and infected seedlings. (J) Characteristic symptoms of *C. lunata* in the root, collar and the primary leaves of a weakly-infected seedling.

coat, 40 to 75% on cotyledons and 25 to 65% on the embryonal axis of weakly and heavily symptomatic seeds of samples 3 and 18, respectively (Figure 2). Another study in histochemical localisation was cleared whole-mount preparations, infection detected as 40, 20% in the seed coat and 20, 20% in cotyledons of asymptomatic seeds of the two samples, respectively.

Broad, dark brown, septate, branched inter- and intra-

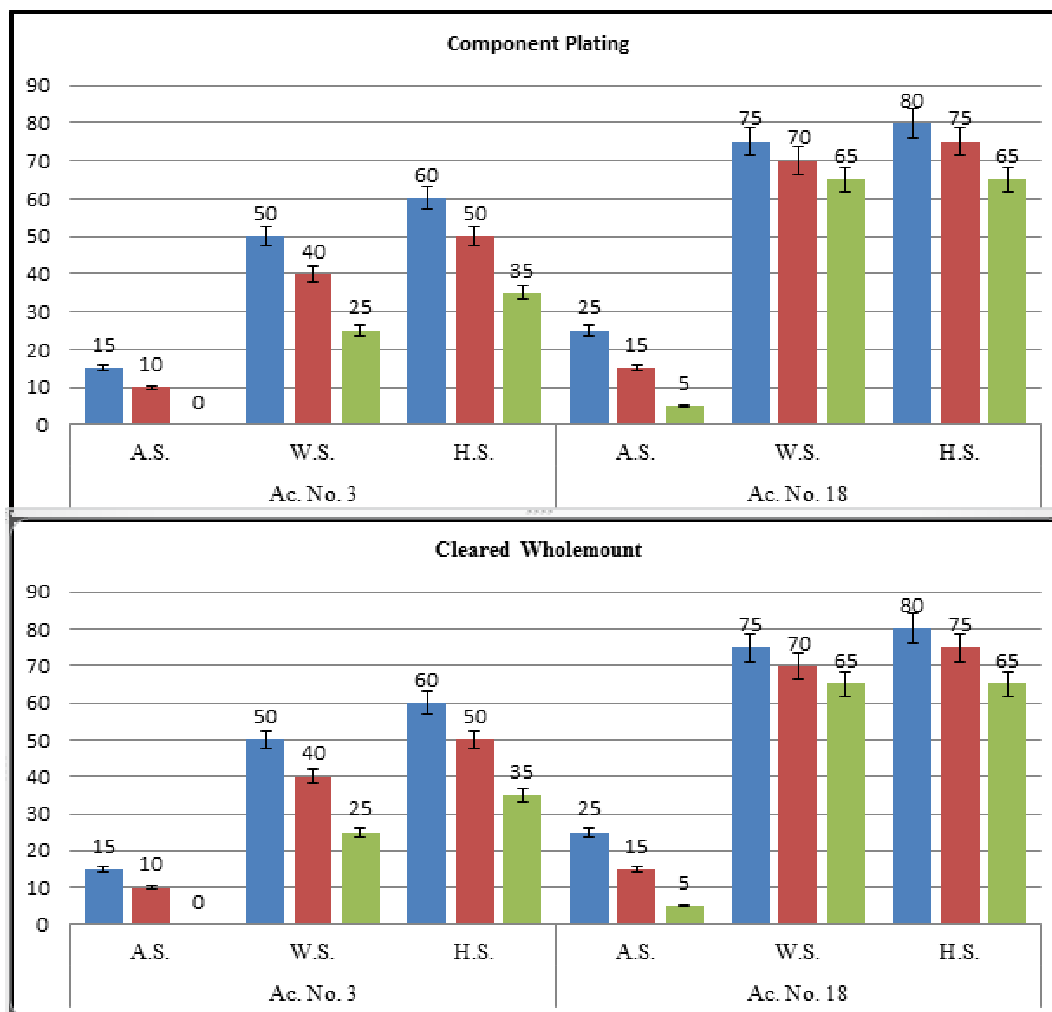

Figure 2. Percent incidence of *Curvularia lunata* in various components of seed and its cleared whole mount preparation (using seed sample with accession number 3 and 18 respectively) showing error bars with percentage. Note: Blue- Seed Coat, Red- Cotyledons and- Green Embryonal axis. Ac. No.= Accession Number of the seed samples collected during study; A S.= Asymptomatic; Symptomatic(W S. = Weakly Symptomatic and H S. = Heavily Symptomatic).

cellular mycelium was detected in symptomatic seeds (with weak and heavy infection). It varied from 60 to 100% in seed coat, 60 to 80% in cotyledons and 50 to 80% in embryonal axis in two samples, respectively (Figures 2 and 1B).

In localisation studies of pathogen in seed, hand-cut (Figure 1C to D) and microtome sections (Figure 1E to H) revealed infection with pathogen. Five seeds each of the two samples revealed that, asymptomatic seed's mycelium was not observed in the deeper tissues of the seed. Mycelial fragments and hyphal parts were observed in epidermal and subepidermal cells of the seed coat. Whereas weakly symptomatic seed's mycelium, ramified the layers of the seed coat and invaded into cotyledons and rarely hyphal bits seen in embryonic axis (Figure 1A to H). Seed coat cells became irregularly elongated and disfigured, showing the disintegration of the cell wall.

In heavily symptomatic seeds, characteristic inter- and intracellular mycelium was detected in all seed components. Both the cotyledons were compressed, showing necrotic regions and lytic cavities. The cells of the seed coat and cotyledons become enlarged, disfigured, deformed and depleted. Seed coat and cotyledon cells were dead, containing brown pigmented cytoplasm filled with hyphal fragments (Figure 1F).

Due to heavy infection, the disintegration of cells resulted in the formation of lysogenic cavities. Dense hyphal networks were observed in and on the cells of the embryonic axis (Figure 1G). The spread of hyphae was detected from the hilar region to epidermal cells of seed coat followed by aleurone layer and cotyledonary cells of

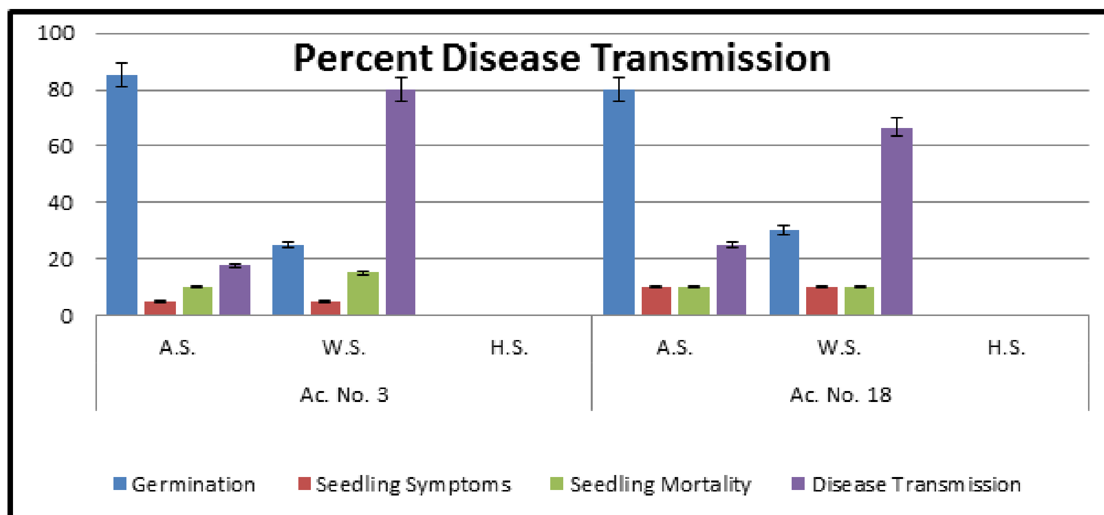

Figure 3. Phytopathological effects of *Dalbergia sissoo* seeds naturally infected with *Curvularia lunata* in Water-agar seedling symptom test (WASST) and pot experiment showing error bars with percentage.

the embryo (Figure 1F).

During transmission studies in standard blotter method, germination was started after one day of incubation as 85, 80% in the two samples studied and on the 12th day, it declined to 25, 30% of sowing in asymptomatic seeds and weakly symptomatic seeds. The seedling exhibited high pre- and post-emergence mortality; whereas, heavily symptomatic seeds failed to germinate leading to 100% pre-emergence mortality due to fungal growth and seed rotting. For water agar seedling symptom test, germination was 95, 90% in asymptomatic seeds and 15, 25% in weakly symptomatic seeds on the 14th day of sowing of both samples (Figure 3).

Total pre- and post-emergence loss and disease transmission varied from 15, 15, 17.6, 25.0% and 45, 35, 80.0, 66.6% in asymptomatic and weakly symptomatic seeds of the two samples, respectively (Figure 3). Initial signs of disease symptoms appeared on the 4th day of sowing; the root-shoot transition region became brown from where it spreads towards the root tip. The roots were poorly developed and stunted. Subsequently on 8 to10th day, brown streak developed on transition region, brown necrotic spots appeared on the cotyledon leaves and finally rotting occurred in the whole seedling (Figure 1I and J).

DISCUSSION

Study on histopathological and phytopathological effects of seed-borne nature of *C. lunata* on *Dalbergia sissoo* Roxb was found to be limiting but the present study gave a comprehensive account by the review. The pathogen was found to be externally- and internally seed- borne, having characteristic feature that can be recognised during dry seed examination and was confirmed by histopathological and phytopathological studies. Similar observations in seed and seedling rotting were reported in sorghum due to *C. lunata* (Rastogi et al., 1990; Kamaluddeen and Lal, 2013). Cui and Sun (2012) reported the incidence of *C. lunata* in *Nelumbo nucifera* while Singh (2002) reported similar symptoms with high pre- and post-emergence losses and failure in germination, due to heavy infection. The pathogenicity was confirmed by artificial inoculation of healthy plant parts which resulted in the appearance of disease. Such studies were supported by a research of Ashour and El-Kadi (1958).

Another study reported *Acacia nilotica* as a new host for *C. lunata* which studied the cultural and morphological characters of the pathogen (Singh and Jamaluddin, 1995). Pathogenicity of the fungus was proved by spraying eight days old suspension of *C. lunata* on leaves. In a study of some dominant seed borne fungi of a rice variety, twenty dominant fungi were found to be associated by blotter method; agar plate method showed that species of *Aspergillus, Fusarium, Alternaria* and *Curvularia* are the dominant genera affecting seed germination and seedling vigour (Islam and Borthakur, 2012). The study suggested that shisham is an important multipurpose tree with great economic importance. But this tree has been devastated by die back disease due to four fungi (*Fusarium solani, Botryodiplodia theobromae, C. lunata* and *Ganoderma lucidum*). Ahmad et al. (2013) evaluate the potential role of these fungi in shisham die back disease. The seeds of *Dalbergia sissoo* were collected from 10 locations in Bihar. Seeds were placed on PDA medium and observed for the growth of fungus after 5 to 10 days of incubation.

This result indicates six genus of fungi *viz*. *Alternaria*

alternata, Penicillium citrinum, Helmenthosporium sp., *C. lunata, Geotricum* sp, respectively (Naz et al., 2015). The present study revealed that the infected seeds are asymptomatic or symptomatic (weakly and heavily infected). *C. lunata* was mostly found to be associated with seeds with black discolorations along with dull appearance, and was both externally and internally seed-borne. The role of seed-borne inoculums of *C. lunata* in disease transmission has been investigated in shisham (Bhowmick and Vardhan, 1981), in Sudan grass (Komoto and Hori, 1983) and Pearl millet (El-Zayat et al., 1990). However, its pathogenicity was confirmed by artificial inoculation in healthy host tissue, which resulted in disease.

During transmission studies, the pathogen moved from seed to seedling/plant which caused a high pre- and post-emergence loses, and symptoms appear as, brown streak transition region and brown necrotic spots primary leaves. Later the whole seedling got rotten and was succumbed to death. According to a study, *Curvularia* sp. is a highly pathogenic fungus with main seedling rot, causing agent in Sudan grass (El-Zayat et al., 1990; Rajput et al., 2010). Similar results had been reported by another study on *D. sissoo*, indicating a reduction in seed germination percentage (50.00%) in soil infested with *F. solani.* Seedling mortality was 93.33% followed by soil infested with *R. solani* (60.00%) with a mortality rate of 66.66% and *C. lunata* of 70.00%. The seedling mortality rate of 42.85% is compared to that of *F. moniliformi* and *F. oxysporum*, respectively (Nadkarni, 1954).

Conclusion

The pathogen *C. lunata* is both externally and internally seed-borne. The internal inoculum affected seed germination, and viability which caused high total (pre- and post-emergence) losses. This pathogen was transmitted from seed to seedling, causing heavy losses to tree plantation.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

REFERENCES

Ahmad BI, Khan RA, Siddiqui MT (2013). Incidence of dieback disease following fungal inoculations of sexually and asexually propagated shisham (*Dalbergia sissoo*). For. Pathol. 43:77-82.

Anonymous (1952).The Wealth of India. Raw Materials, Vol. HI,D-E, CSM New Delhi. http://www.niscair.res.in/

Ashour WE, El-Kadi MM (1958). Cultural studies of Fusarium semitectum *Alternaria tenuis* and *Rhizoctonia solani* which cause damping – off of tomato seedlings. A'in Shams Sci. Bull. 3:57-68.

Bhowmick BN, Vardhan V (1981). Antifungal activity of some leaf extracts of medicinal plants on *Curvularialunata*. Phytopathologicalnote. Indian Phytopathol. 34:385-386.

Champion HG, Seth SK (1968). A Revised Survey of the Forest Types of India, Manager of Publication, Delhi.

Cui RQ, Sun XT (2012). First Report of *Curvularia lunata* Causing Leaf Spot on Lotus in China. Plant Dis. 96(7):1068-1069.

El-Zayat MM, Mansour IM, Moursy MA, Abdel-Fattach MND (1990). Stalk and root rots of certain cereal forage crops in Egypt. Ann. Agric. Sci. Moshtohor 28(3):1525-1537.

Islam NF, Borthakur SK (2012). Screening of mycota associated with Aijung rice seed and their effects on seed germination and seedling vigour. Plant Pathol. Quar. 2:75-85.

ISTA (1990). International rules of seed testing. Seed Sci. Technol. 18:299-513.

Kamaluddeen SS, Lal AA (2013). A New Blight Disease Of Rice Caused By Curvularia Lunata From Uttar Pradesh. Int. J. Agric. Sci. Res. 3(5):13-16.

Komoto Y, Hori M (1983). Factors affecting development of Curvularia leaf spot of sudan grass. Bull. Chugoker Natl. Agric. Exp. Stn. 20:15-23.

Marshall BJ, Armstrong JA, McGechie DB, Glancy RJ (1985). Attempt to fulfil Koch's postulates for pyloric Campylobacter. Med. J. Aust. 142(8):436-439.

Nadkarni KM (1954). Indian Material Medico, 3rd edition, Popular Book Depot, Bombay and Dhootapapeshwar Prakashan Ltd.

NAS (1983). Firewood Crops: Shrub and Tree Species for Energy Production, Vol. 11, National Academy of Science Press, Washington, D.C.

Naz H, Naz A, Pandey A (2015).Studies on Fungi Associated with Dalbergiasissoo Roxb.ex. DC, Lambert Academic Publishing.

Nelson RR, Haasis FA (1964). The Perfect Stage of Curvularia lunata. Mycologia 56(2):316-317.

Rajput NA, Pathan MA, Rajput Q, Jiskani MM, Lodhi M, Rajput SA, Khaskhal MI (2010). Isolation of fungi associated with shisham trees and their effect on seed germination and seedling mortality. Pak. J. Bot. 42:369-374.

Rastogi R, Singh T, Singh D (1990).Infection of *Curvularialunata*in all sorghum seeds. J. Indian Bot. Soc. 69:71-73.

Sharma P, Singh N, Verma OP (2012). First report of Curvularia leaf spot, caused by *Curvulariaaffinis* on *Dalbergiasissoo.* For. Pathol. 42:265-266.

Singh B, Jammaluddin (1995). *Acacia nilotica*:new host of *Curvularia.* Indian Forester 121(2):161.

Singh R (2002). Seed and Seedling Diseases of Pearl Millet and their Control, Ph.D. Thesis, University of Rajasthan, Jaipur.

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Full Length Research Paper

New records of basidiomycetous macrofugi from Kurdistan region - Northern Iraq

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This study was carried out within February to June, 2015 to 2016 on macrofungi samples collected from different localities within Iraqi Kurdistan region - Northern Iraq. This mountainous region is rich in forest trees with diverse groups of shrubs and herbs and is expected to support the growth of many macrofungal species. However, this part of Iraq is still unexplored from macrofungal point of view. In this paper seven basidiomycetous macrofungal species from seven genera , six families and two orders : *Inocybe flocculosa, Pleurotus nebrodensis, Psathyrella spadiceogrisea, Schizophyllum commun*e, *Volvopluteus gloiocephalus* (Agaricales), *Lentinus tigrinus* and *Trametes trogii* (Polyporales) were reported from Iraqi Kurdistan. These macrofungal species are recorded for the first time from Iraq.

Key words: Macrofungi , Agaricales , Polyporales , Iraqi Kurdistan.

INTRODUCTION

Macrofungi (or macromycetes) are fungi that produce fruiting bodies visible to naked eye (Mueller et al., 2007). Macrofungi are Basidiomycota or Ascomycota and most of them are saprotrophic or mutualistic (mycorrhizal) but some are plant pathogens (Mueller et al., 2007; Devi and Shrivastava, 2016). Beside their role in ecosystem processes (decomposers of dead wood, bioremediation and biocontrol agents, mycorrhizal organisms), macrofungi serve as food ,medicine and producers of pharmaceutical active compounds and many other benefits (Redhead, 1997; De Silva et al., 2013) . Out of 1.5 million species of fungi estimated in the world, only 21,679 macrofungal species (that is, 1.5% of all known fungal species) have been described (Mueller et al., 2007) .

Kurdistan of Iraq is a mountainous region situated at the northern and north eastern parts of Iraq, varying from some 500 to 800 m in altitude in the lowest valleys and 2000 to 3600 m at the summits of the highest ranges (Lahony, 2013).

The Iraqi Kurdistan region (36° 11′ 0″ N, 44° 0′ 0″ E) comprises three governorates, Erbil, Suliamaniya and Duhok. It is bordered by Syria to the west, Iran to the east, and Turkey to the north, lying where fertile plains meet the Zagros Mountains. It is traversed by the Sirwan River and the Tigris and its tributaries, the Great Zab and

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the Little Zab.

Kurdistan of Iraq covers about 40,000 Km^2 of Iraq. It is with a cold winter (December – March or April) and relatively high rainfall upwards to 800 mm and the mountains above approximately the 1800 m level are snowbound for several months and snows often falls in the valleys, while the summer though hot and dry, is comparatively of shorter duration (June to September) than on the other parts of Iraq (May to October). These factors contribute to richer biodiversity situation especially the floral components (Lahony, 2013). Despite its biogeographic importance, Northern Iraq is still unexplored from macrofungal standpoint. However, surveys on macrofungi were reported from some countries bordering Iraq like Turkey (Akata et al., 2014; Güngör et al., 2013; Güngör et al., 2015; Sesli and Denchev, 2008), Iran (Amoopour et al., 2016; Ghobad - Nejhad and Kotiranta, 2008; Ghobad-Nejhad and Hallenberg, 2012), Jordan (Saba, 1991) and Saudi Arabia (Abou - Zeid and Altalhi, 2006) . This paper deals with seven basidiomycetous macrofungi new to Iraq.

MATERIALS AND METHODS

Macrofungi samples were collected from different localities within Kurdistan region in the north of Iraq during February to June, 2015 to 2016. These localities are Sami Abdul - Rahman Park (36° 11´ 32.6328" N 43° 59´ 7.3356" E, elevation 394 m) and Qandil mountains (36° 32´ 28" N 44° 59´ 46" E , elevation 3587 m), Erbil district; Chuwarta (35° 43' N 45° 34' E, 20 Km NE Suliamaniya, elevation 1361 m), Dukan lake (36° 08′ N 44° 55′ E, 60 km NW of Suliamaniya, elevation 515 m) and Tawela (35° 12 N 46° 10´ E , elevation 1500 m), Suliamaniya district; Amadia (37° 05′ 33″ N 43° 29′ 14″E , 70 km N of Duhok, elevation 1202 m), Amadia district. Habit (solitary, gregarious or other growth form) and habitat (host or substratum) of the samples with season of fruiting body appearance were recorded and samples were photographed in their natural habitats. Macroscopic features (including features such as cap size, shape, color and surface texture; gills color, attachment to stipe (if present); stipe size, color and surface texture) and microscopic features (including characters like basidia size and their spore number, spore shape, size and color and presence or absence of cystidia and their shapes and types) of macrofungi were reported. Cotton blue in lactophenol was used for light microscopy. Macrofungi were identified according to literatures, keys and monographs (Gargano et al., 2011; Justo et al., 2011; Kuo, 2003; Kuo, 2011; Laursen et al., 2013; Phillips, 2013; Ryvarden and Gilbertson, 1993; Senthilarasu, 2015; Watanabe, 2010). All samples were deposited in Biology Department, College of Sciences, Tikrit University.

RESULTS AND DISCUSSION

During the survey of different localities of Iraqi Kurdistan region, Northern Iraq, seven basidiomycetous macrofungal species, *Inocybe flocculosa, Pleurotus nebrodensis, Psathyrella spadiceogrisea, Schizophyllum commune, Volvopluteus gloiocehalus (*Agaricales), *Lentinus tigrinus* and *Trametes trogii* (Polyporales) were reported. These fungi are reported for the first time from Iraq. Their description and distribution are given as follows:

Kingdom: Fungi
Subkingdom: Dikarya
Phylum: Basidiomycota
Subphylum: Agaricomycotina
Class: Agaricomycetes
Subclass: Agaricomycetidae
Order: Agaricales
Family: Inocybaceae
Species: *Inocybe flocculosa* (Berk) Sacc.
Fruiting body: Cap 20 to 25 mm broad, tiny, fibrous, bell or convex with light umbonate, pale brown, surface tomentose to squamose appear lighter than the ground color; gills attached to the stipe, pale brown, crowded; stipe 40 to 60 mm long, 3.0 to 3.8 mm wide, cylindrical, surface cream colored, pruinose, solid, bent at the bulbose base, central; ring and volva absent (Figure 1). Microscopic feature: Basidium 20 - 25 × 6.25 - 7.5 µm, 4 - spored, spores 8.0 - 11.25 × 5.5 - 6.5 µm, almond, smooth, light brown color; cheilocystidia and pleurocystidia similar 90.6 - 112.0 × 20 - 25 µm, hyaline, fusiform, apically with light brown crystals (Figure 2).

Gregarious among leaf litter in mixed forest of *Quercus* spp. and other tree species in Chuwarta, Suliamanyia district, inedible, March to April. *Inocybe flocculosa* was reported from Japan (Kobayashi and Courtecuisse, 1993), India (Cimap, 2005) and Turkey (Sesli and Denchev, 2008; Solak et al., 2009). Reports are not available on this species from Iran and Arab countries bordering Iraq.

Order: Agaricales
Family: Pleurotaceae
Species: *Pleurotus nebrodensis* (Inzenga) Quel.
Fruiting body: cap 38 to 142 mm broad , 60 to 110 mm high, fleshy, initially hemispherical to convex with inrolled margin, then flattens and finally depressed at centre with uplifted wavy margin, smooth, cracking a part at maturity; gills decurrent, whitish at first then turn white yellowish or creamy or light brown, crowded, edges smooth; stipe 35 to 83 mm long, 17 to 38 mm wide, cream or whitish, central or excentric , unequal cylindrical, often bent, surface with longitudinal grooves, solid; ring and volva absent (Figure 3). Microscopic features: Basidium 45 - 55 × 8 - 10 µm , 4 - spored , spores 9.0 - 12.5 × 5.0 - 7.5 µm, hyaline, cylindrical, smooth; cheilocystidia abundant 45 - 50 × 7.5 - 9.0 µm, granular capitate (Figure 4).

Solitary or gregarious on plants debris, edible, Haw Mountain, Amadia district and Qandil Mountain, Erbil district, April to May. *Pleurotus nebrodensis* was reported from Turkey (Sesli and Denchev, 2008) and other countries like Spain (De Román and Boa, 2004) and Italy (Venturella et al., 2002). This species was declared by IUCN as critically endangered species in 2006

Figure 1. *Inocybe flocculosa*. a – c: fruiting body.

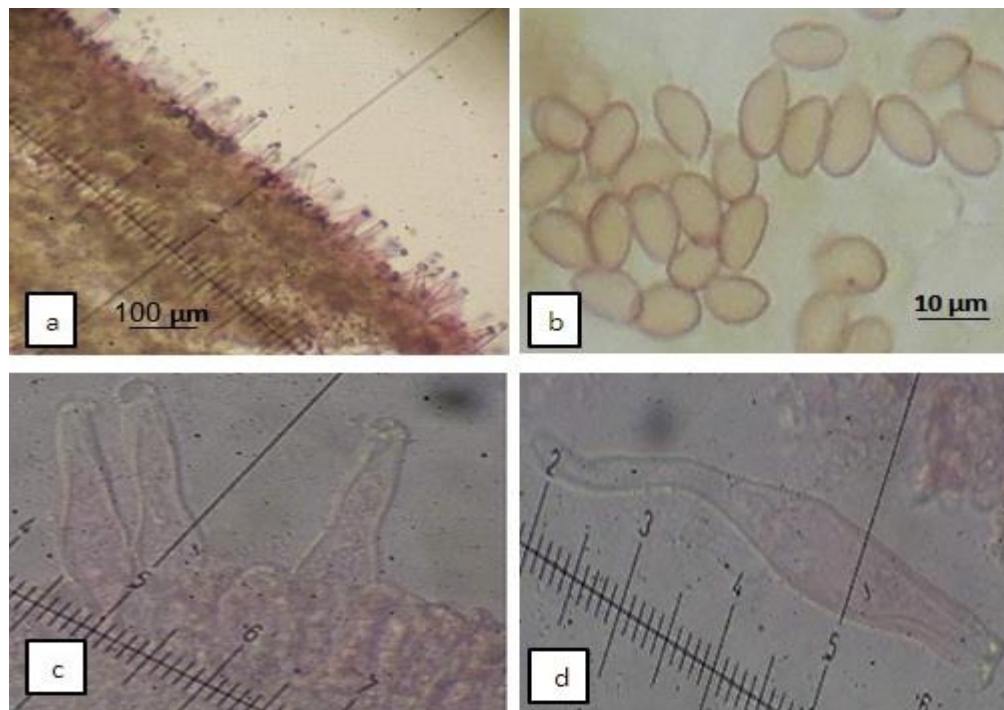

Figure 2. *Inocybe flocculosa. a,* hymenium; b, spores; c, d, cystidia (1 line = 2.5 µm).

(Venturella, 2006).

Order: Agaricales
Family: Psathyrellaceae

Species: *Psathyrella spadiceogrisea* (Schaeff.) Maire. Fruiting body: Cap 10 to 30 mm broad, 5 to 10 mm high, somewhat fleshy, conical or convex, smooth, medium brown; gills attached to the stipe, dark brown; stipe 10 to

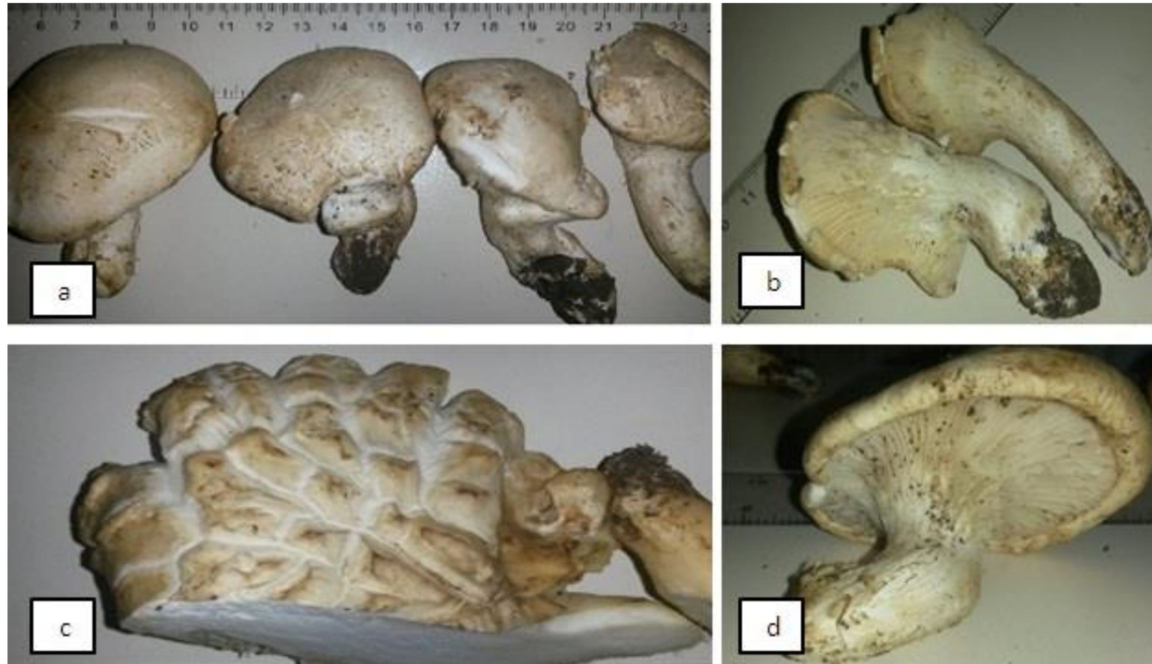

Figure 3. *Pleurotus nebrodensis.* a - d, fruiting body. Note the bent stipe in d.

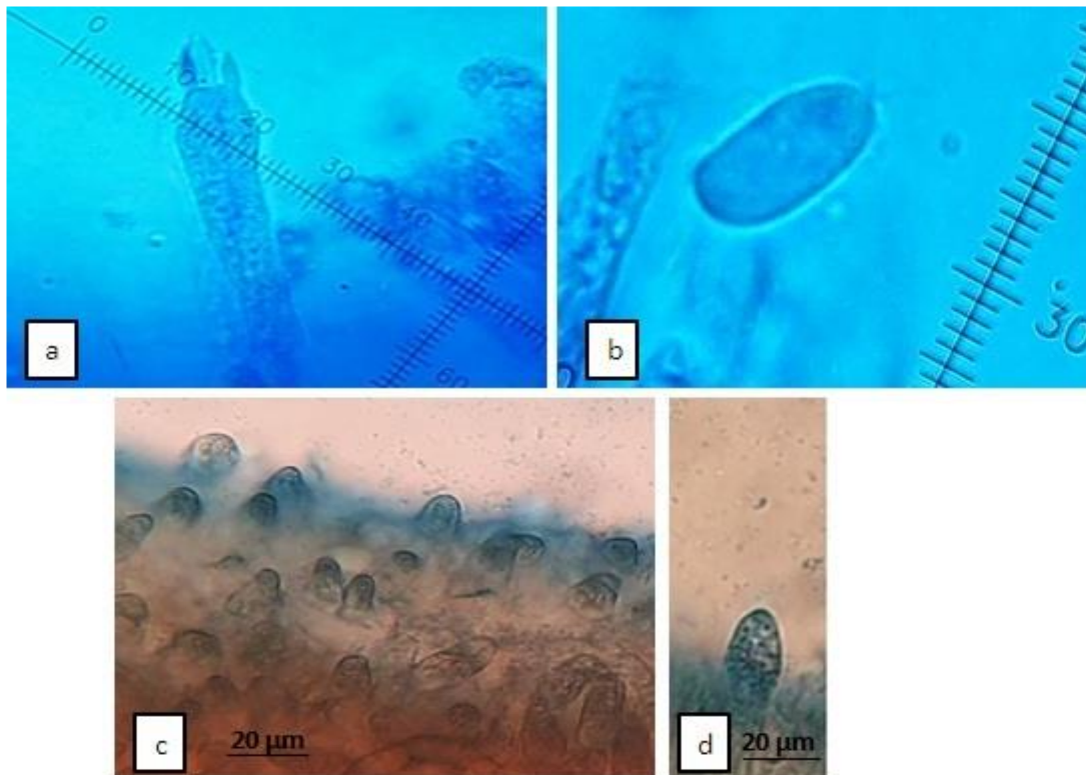

Figure 4. *P. nebrodensis. a*, basidium; b, spore; c, cheilocystidia - surface view; d, granular capitates cystidia . a, c, d: 1 line = 2 µm.

50 mm long, 10 to 12 mm wide, unequal cylindrical, central, hollow, whitish, appear dark brown at the upper part near the cap, without ring and volva (Figure 5).

Microscopic features: Basidium 20 - 25 × 10 - 12 µm, 4

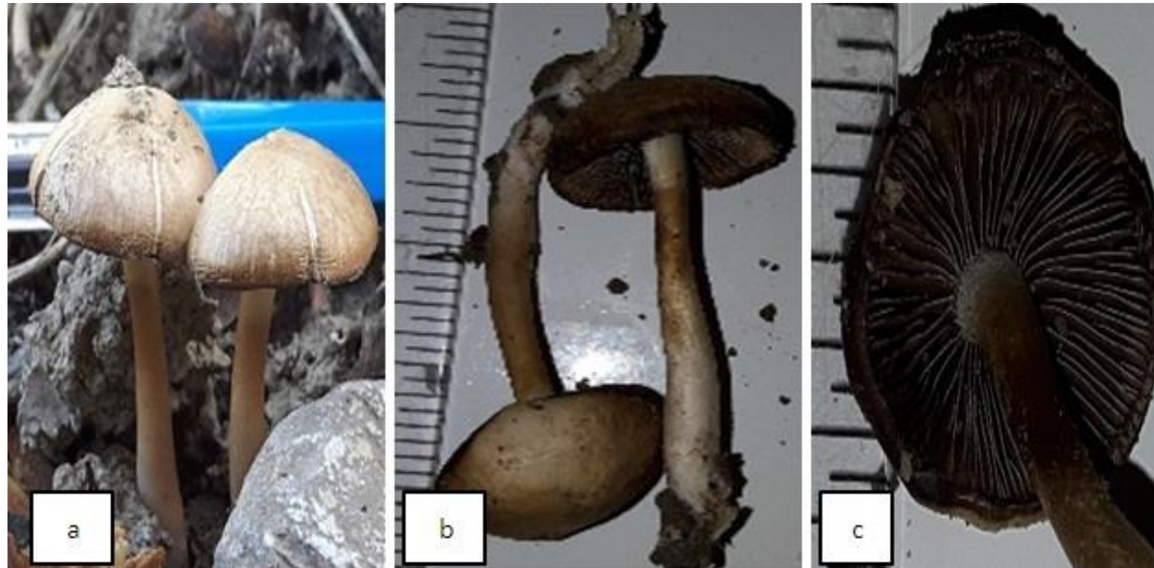

Figure 5. *Psathyrella spadiceogrisea*. a, fruiting body in natural habitat; b, unequal cylindrical stipe; c, gills - stipe attachment.

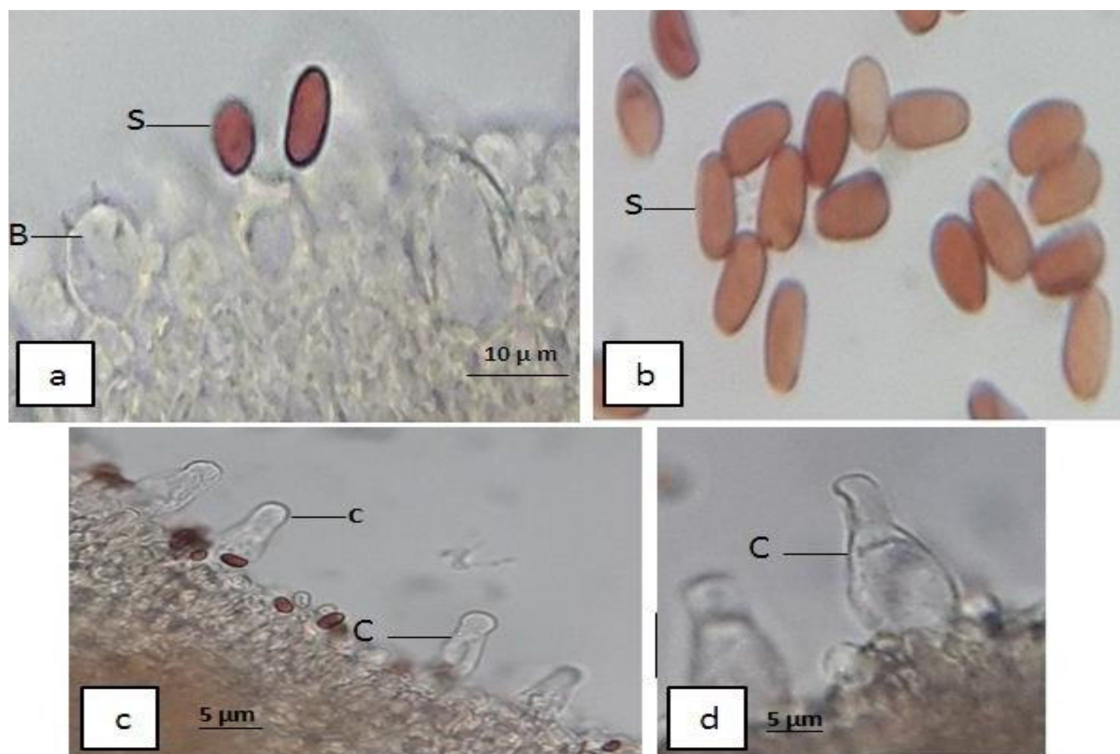

Figure 6. *P. spadiceogrisea*. a, basidium (B) and spores (S); b, spores; c, pleurocystidia - surface view ; d, utriform pleurocystidia (C).

- spored, spores 7 - 10 × 4.0 - 6.5 µm, elliptical with pore, smooth, deep brown; pleurocystidia clavate and utriform to about 50.0 × 12.5 µm; cheilocystidia similar to utriform pleurocystidia (Figure 6) .

Solitary or gregarious on trunks of dead grape (*Vitis vinifera*) trees, inedible, Dukan Lake, Sulaimaniya district, February to March . This fungal species was reported from Italy (Perini et al., 1999), Austria (Pidlich - Aigner et

Figure 7. *Schizophyllum commune*. a, b, fruiting body in natural habitat; c, fruiting body on burned trunk.

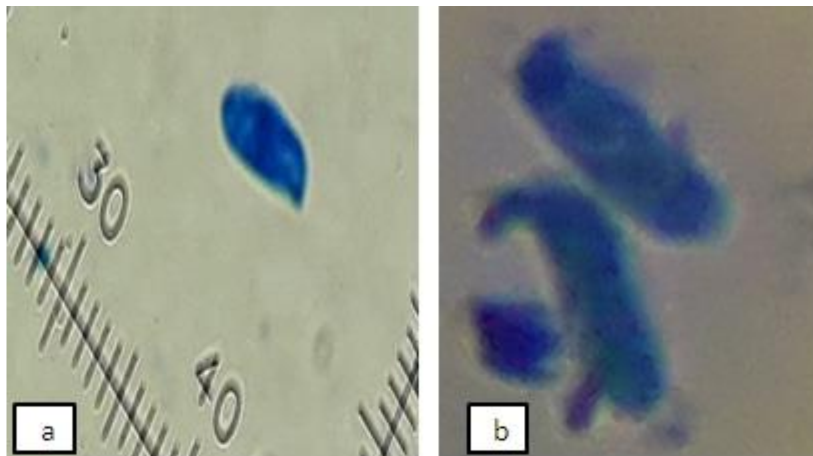

Figure 8. *S. commune*. a, spore; b, spores magnified . 1 Line = 1 μm.

al., 2001), Czech Republic and Slovakia (Vašutová, 2006), Nordic countries (Larsson and Örstadius, 2008), Turkey (Doğan et al., 2007; Sesli and Denchev, 2008) and Cameron (Kinge et al., 2013).

Order: Agaricales
Family: Schizophyllaceae
Species: *Schizophyllum commune* Fries
Fruiting body: Cap 10 to 50 mm broad, tiny, sessile or on short stem, fan shaped or shell shaped, irregular, depending on where it attached to the trunk, white to light grayish or tan, upper surface covered with dense hairs; gills grayish, narrow, diffuse from the attachment point, split longitudinally and they curl back to protect the hymenium during dry weather (Figure 7). Microscopic features: basidium 4 - spored; spores 5.5 - 7.0 × 0.9 - 3 .0 μm, cylindrical, curved in one end, smooth, hyaline; clamp connections present; cystidia absent (Figure 8).

Overlapping or gregariously on dead or living *Prunus domestica* and *P. armeniaca* trees, edible, Dukan Lake, Sulaimaniya district, March to April. *Schizophyllum commune* was reported from Turkey (Afyon et al., 2005; Sesli and Denchev, 2008), Iran (Ghobad-Nejhad and Wallenberg, 2012) and other countries like Brazil (Groposo and Loguercio, 2005).

Figure 9. *Volvopluteus gloiocephalus*. a, b, fruiting body in natural habitat with bell shaped cap in a and convex cap in b; c, croweded gills; d , stipe with volva ; e, stipe with white granules .

Order: Agaricales
Family: Pluteaceae
Species*: Volvopluteus gloiocephalus* (DC.) Vizzini, Contu & Justo
Fruiting body: Cap 76 to 90 mm broad , fleshy , bell shaped at early stage becoming convex or flat in age with umbonate, radialy streaked with apprised pale grayish fibrils, smooth, shiny, whitish to creamy; gills free from the stipe, broad, thick, crowded and have a pink color; stipe 124 to 129 mm long, 10 to 12 mm wide, solid, easy to separate from the cap, cylindrical tapering slightly to top, pruinose, ring absent; volva: 21 to 25 mm long, membranous, sac - like (Figure 9). Microscopic features: Basidium 22.5 - 40.0 × 10.0 - 12.5 µm, 4 - spored; spores 10.0 - 17.5 × 8.0 - 11.25 µm, elliptical with smooth wall , pinkish brown color; both pleurocystidia (50 - 75 × 10 - 15) µm and cheilocystidia clavate; clamp connections absent (Figure 10).

Solitary, scattered on soil, edible, Sami Abdul - Rahman Park, Erbil district, March to April. *Volvopluteus gloiocephalus* was reported from Turkey (Atila and Kaya, 2008; Kaya, 2015) and Iran (Fadavi and Abbasi, 2015).

Order: Polyporales
Family: Polyporaceae
Species: *Lentinus tigrinus* (Bull.) Fr.
Fruiting body: Cap 42 to 140 mm broad, fleshy, convex at early stage with blackish brown then becoming funnel shaped and white yellowish color with a somewhat wavy margin, upper surface covered with dense blackish brown hairs or scales which become scattered and crowded over the center in age; gills - like decurrent, white to yellowish white, crowded, toothed; stipe 48 to 62 mm long, 6 to 12 mm wide, yellowish white, centric, cylindrical, attenuate downwards into blackish root - like extension, covered with blackish brown hairy scales, dark at the base, often bent, hollow; ring and volva absent (Figure 11) . Microscopic features: basidium 18 - 20 × 4.5 - 5.0 µm , 4 - spored; spores 6.25 - 10.0 × 2.5 - 3.75 µm, narrow cylindrical or fusiform, hyaline, smooth; clamp connections present; cystidia absent (Figure 12).

Gregarious on dead *Pistacia atlantica* trees (Habbat Khadra in Arabic, Qazwan in Kurdish), localy edible, Haw mountain, Amadia, Amadia district, April to June. This

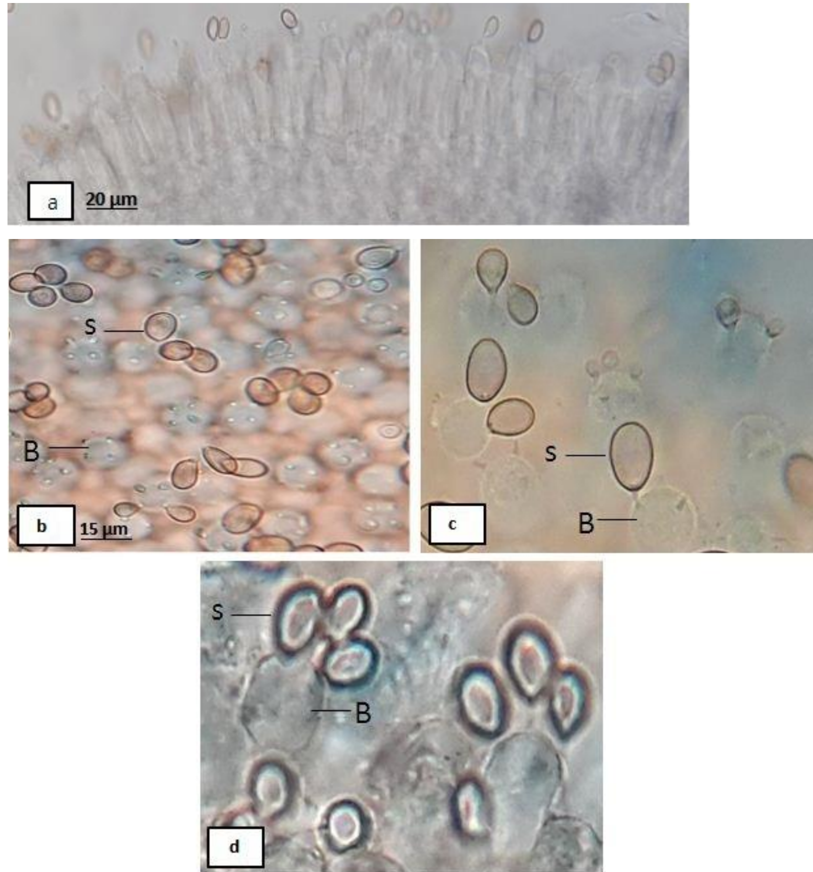

Figure 10. *V. gloiocephalus*. a, hymenium; b, basidia (B) and spores (S) surface view; c, d, basidia and spores magnified.

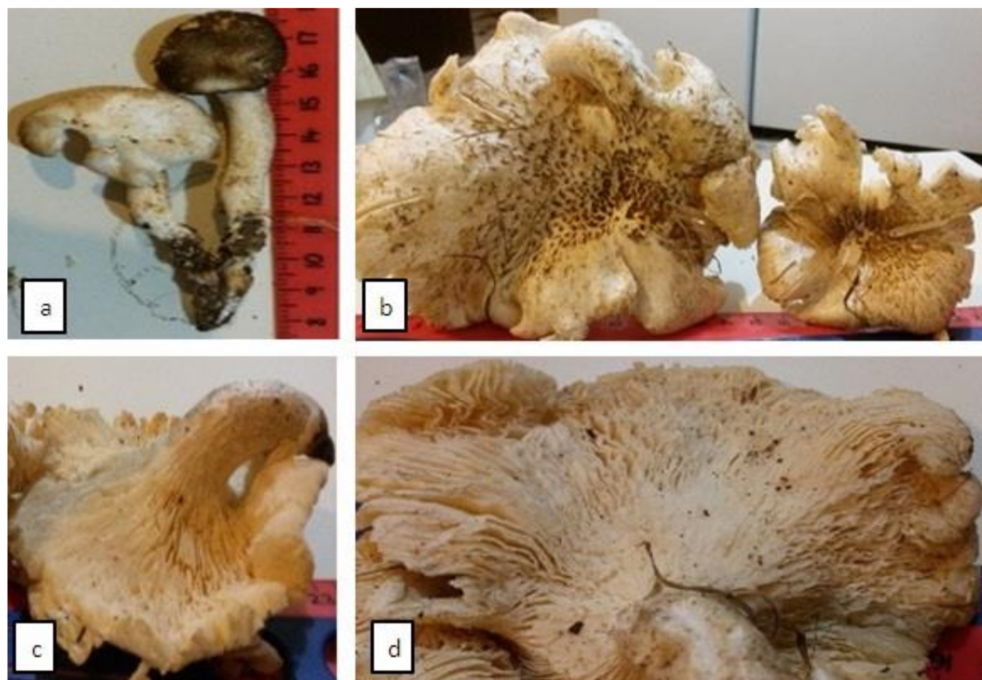

Figure 11. *Lentinus tigrinus.* a, young fruiting body with blackish cap; b, cap upper surface covered with blackish brown hairs; crowded hairs over cap center; c, hairs on the stipe; d, toothed gills .

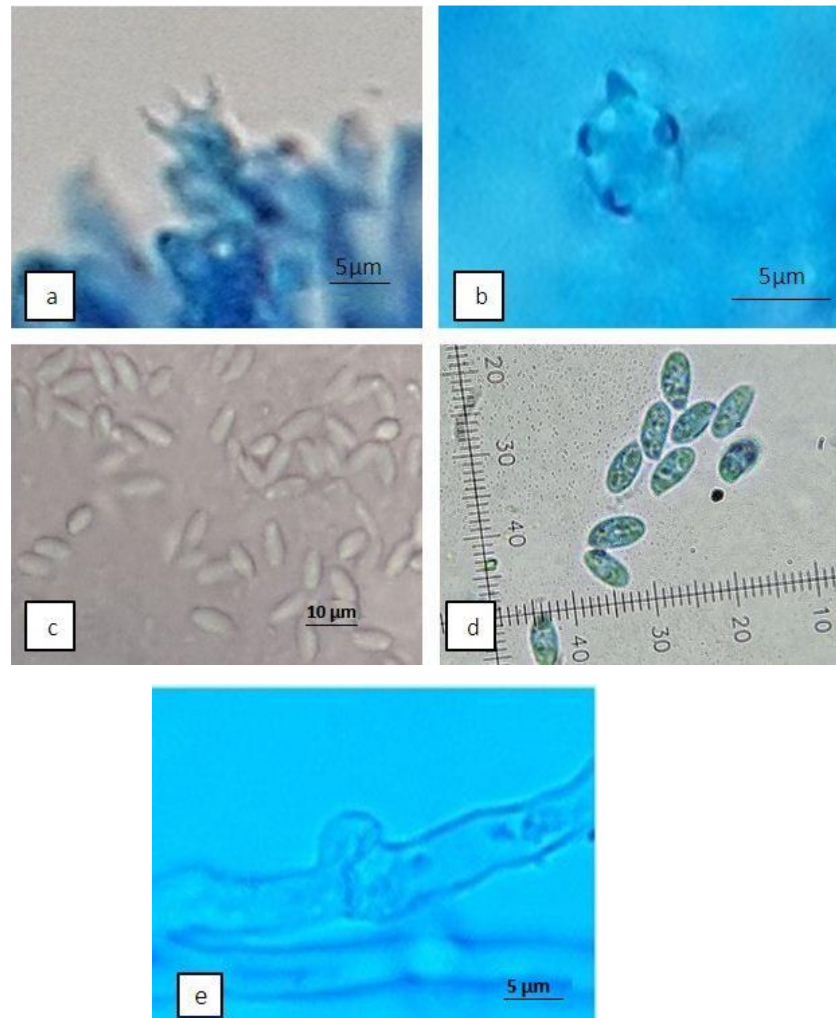

Figure 12. *L. tigrinus.* a, basidium in section; b, basidium in surface view with four sterigmata; c, spores unstained; d, spores stained ; e, clamp connection.

fungus was reported from Turkey (Sesli and Denchev, 2008), south western Nigeria (Adejumo and Awosanya, 2005), Thailand (Karunarathnas et al., 2011), Philippine (Dulay et al., 2014; Dulay et al., 2012), Pakistan (Razaq and Shahzad, 2015) and India (Senthilarasu, 2015; Sharma and Atri, 2015).

Order: Polyporales
Family: Polyporaceae
Species: *Trametes trogii* Berk.
Fruiting body: Cap 46 to 163 mm long, sessile, effused or resupinate, snowy white or cottony colored flesh. In maturity, the color turns to cream - buff or brown - buff, the upper surface covered with roughen brown hairs and the texture of the fungus becomes tough and wooden in age and shows the surface of the cap divided into zonate and sharp edges, flesh brown, continuous with tubular layer and characterized by the inability to separate the tubes from each other and ends at apex with circular brown pores (Figure 13). Microscopic features: Basidium 19 - 22 × 5.5 - 7.5 µm wide, 4 - spored, spores 10.0 - 12.5 × 2.5 - 5.0 µm, cylindrical, hyaline; hyphal system, trimitic, generative hyphae with clamp connections, skeletal hyphae with non - septate and binding hyphae that is frequently branching; cystidia absent (Figure 14).

Gregarious to cluster on soil in orchards and on dead *Populus* trees, inedible, Amadia, Amadia district, April to June. *Trametes trogii* was reported from Turkey (Sesli and Denchev, 2008) and warmer parts of Europe (Ryvarden and Gilbertson, 1993).

Conclusion

It is concluded that the vegetation and climatic conditions make Iraqi Kurdistan an ideal place for growth and development of macrofungi. This region is still unexplored

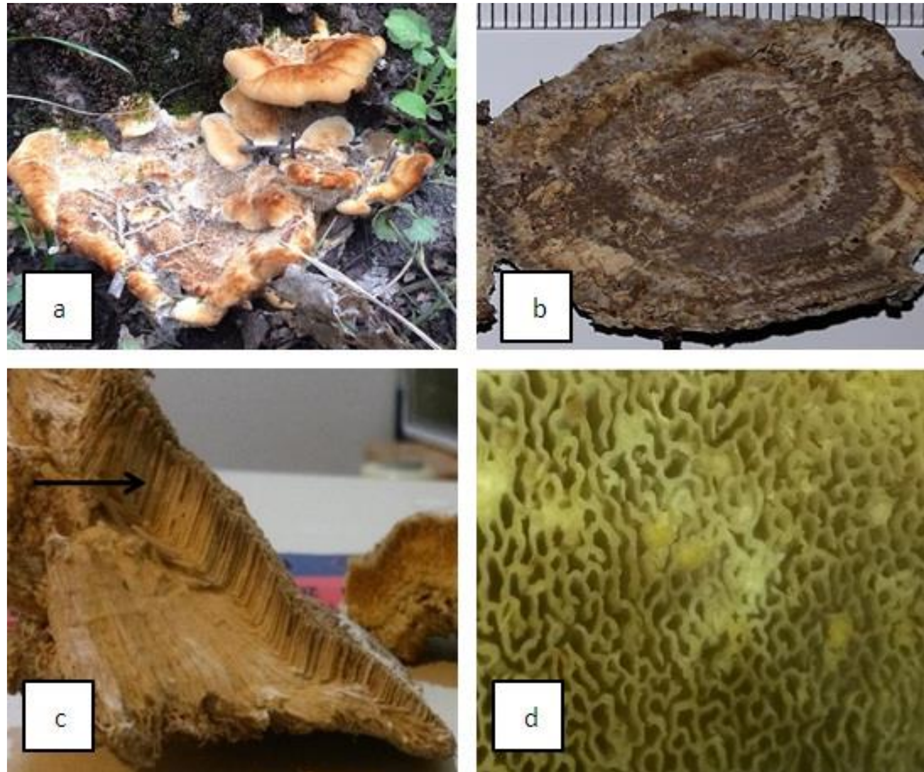

Figure 13. *Trametes trogii.* a, fleshy fruiting body in natural habitat; b, woody fruiting body with zonate upper surface; c, tubular layer of fruiting body; d, porous upper surface.

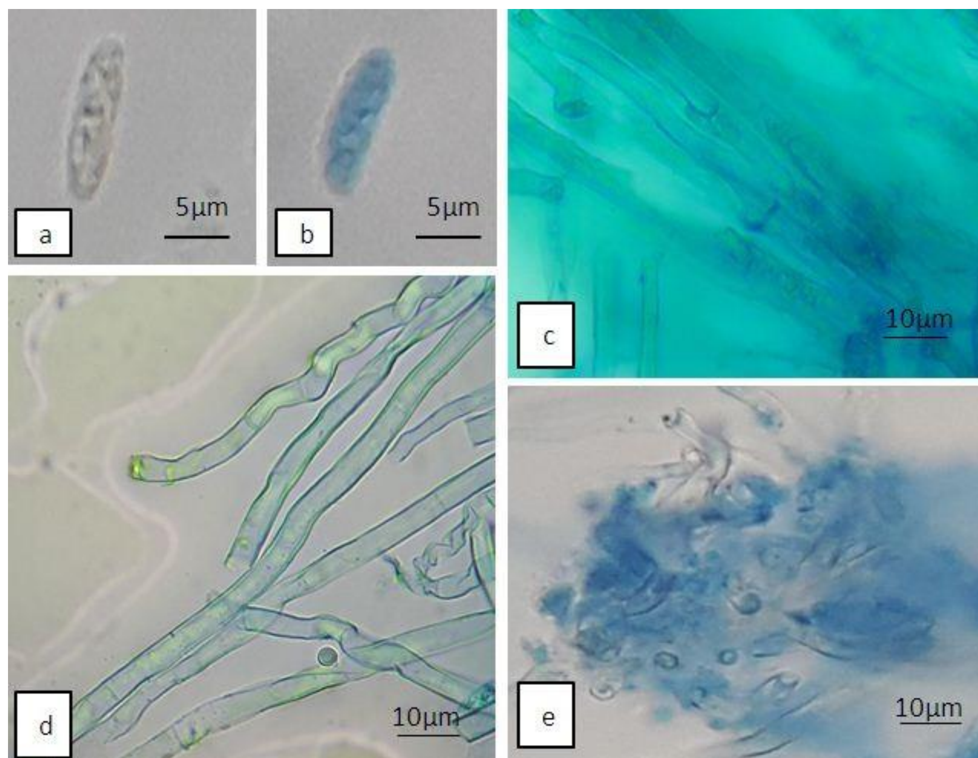

Figure 14. *Trametes trogii.* a, stained spore; b, unstained spore; c, generative hyphae with clamp connections; d, skeletal hyphae ; e, binding hyphae.

from macrofungal point of view. Hence further survey of this group of fungi in this region is of great importance towards creating a checklist of macrofungi in Iraq.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

REFERENCES

Abou-Zeid A, Altalhi A (2006). Survey of some mushrooms in Al-Taif governorate of Saudi Arabia. World J. Agric. Sci. 2(1):1-5.

Adejumo T, Awosanya O (2005). Proximate and mineral composition of four edible mushroom species from South Western Nigeria. Afr. J. Biotechnol. 4(10):1084-1088.

Afyon A, Konuk M, Yagiz D, Helfer S (2005). A study of wood decaying macrofungi of western Black Sea Region, Turkey. Mycotaxon 93:319-322.

Akata I, Uzu Y, Kaya A (2014). Macromycetes determined in Yomra (Trabzon) district. Turk. J. Bot. 38:999-101.

Amoopour M, Ghobad-Nejhad M, Khodaparast S (2016). New records of polypores from Iran, with a checklist of polypores for Gilan Province. Czech Mycol . 68:139-148.

Atila O, Kaya A (2008). Macromycetes of Sarız (Kayseri/Turkey) district. Biol. Divers. Conserv. 6(2):50-54.

Cimap P (2005). Biodiversity of agarics from Nilgiri Biosphere Reserve, Western Ghats. Curr. Sci. 88:1890-1892.

De Román M, Boa E (2004). Collection, marketing and cultivation of edible fungi in Spain. Micol. Aplicada Int. 16:25-33.

De Silva DD, Rapior S, Sudarman E, Stadler M, Xu J, Alias SA, Hyde KD (2013). Bioactive metabolites from macrofungi: ethnopharmacology, biological activities and chemistry. Fungal Divers. 62(1):1-40.

Devi K, Shrivastava K (2016). Diversity of macrofungi in 'Jalukbari reserve forest'of Kamrup District, Assam. Adv. Appl. Sci. Res. 7(1):115-119.

Doğan HH, Öztürk C, Kaşık G, Aktaş S (2007). Macrofungi distribution of Mut province in Turkey. Pak. J. Bot. 38(1):293-308.

Dulay R, Arenas C , Kalaw P, Reyes G, Cabrera C (2014). Proximate composition and functionality of the culinary-medicinal tiger sawgill mushroom, Lentinus tigrinus (Higher basidiomycetes), from the Philippines. Int. J. Med. Mushrooms 16(1): 85-94.

Dulay R, Cabrera E, Kalaw S, Reyes R (2012). Optimal growth conditions for basidiospore germination and morphogenesis of Philippine wild strain of *Lentinus tigrinus* (Bull.) Fr. Mycosphere 3:926-933.

Fadavi S, Abbasi S (2015). A contribution to the identification of agaric fungi of Kermanshah, W Iran (2). Rostaniha 16:1-16.

Gargano ML, Saitta A, Zervakis GI, Venturella G (2011). Building the jigsaw puzzle of the critically endangered Pleurotus nebrodensis: historical collection sites and an emended description. Mycotaxon 115(1):107-114.

Ghobad-Nejhad M, Kotiranta H (2008). The genus Inonotus sensu lato in Iran, with keys to Inocutis and Mensularia worldwide. Ann. Bot. Fenn. 45:465-476.

Ghobad-Nejhad M, Hallenberg N (2012). Checklist of Iranian non-gilled / non-gasteroid hymenomycetes (Agaricomycotina). Mycotaxon 119:494.

Groposo C, Loguercio C (2005). Contribution to the lignocellulolytic fungi (Basidiomycetes) of the Atlantic Rain Forest in Southern Brazil. Mycotaxon 92:103-106.

Güngör H, Alli H, Işiloğlu M (2013). Three new macrofungi records for Turkey. Turk. J. Bot. 37:411-413.

Güngör H, Solak MH, Alli H, Işiloğlu M, Kalmiş E (2015). New records for Turkey and contributions to the macrofungal diversity of Isparta Province. Turk. J. Bot. 39:867-877.

Justo A, Vizzini A, Minnis AM, Menolli N, Capelari M, Rodriguez O, Malysheva E, Contu M, Ghignone S, Hibbett DS (2011). Phylogeny of the Pluteaceae (Agaricales, Basidiomycota): taxonomy and character evolution. Fungal Biol. 115:1-20.

Karunarathnas SC, Yang ZL, Zhao RL, Vellinga EC, Bahkali A, Chukeatirote E, Hyde KD (2011). Three new species of Lentinus from northern Thailand. Mycol. Prog. 10:389-398.

Kaya A (2015). Contributions to the macrofungal diversity of Atatürk Dam Lake basin. Turk. J. Bot. 39:162-172.

Kinge T, Egbe E, Tabi E, Nji T, Mih A (2013). The first checklist of macrofungi of mount Cameroon. Mycosphere 4:694-699.

Kobayashi T, Courtecuisse R (1993). Two new species of Inocybe from Japan. Mycotaxon 46:27-33.

Kuo M (2003). Shizophyllum commune. Retrieved from the Mushroom Expert. Com Web site: http://www.mushroomexpert.com/schizophyllum_commune.html.

Kuo M (2011). Psathyrella spadiceogrisea. Retrieved from the Mushroom Expert. Com Web site: http://www.mushroomexpert.com/ psathyrella _ spadiceogrisea.html.

Lahony SRA, Mohammad MK, Ali HH, AL-moussawi AA, AL-Rasul MSA (2013). Hawraman lowest zone, Kurdistan province north east of Iraq. Bull. Iraq Nat. Hist. Mus. 12:7-34.

Larsson E, Örstadius L (2008). Fourteen coprophilous species of Psathyrella identified in the Nordic countries using morphology and nuclear rDNA sequence data. Mycol Res. 112:1165-1185.

Laursen GA, Ammirati JF, Redhead SA (2013). Arctic and alpine mycology II (Vol. 34). Springer. Science & Business Media.

Mueller GM, Schmit JP, Leacock PR , Buyck B, Cifuentes J, Desjardin DE, Halling RE, Hjortstam K, Iturriaga T, Larsson KH, Lodge DJ (2007). Global diversity and distribution of macrofungi. Biodivers. Conserv. 16(1):37-48.

Perini C, Lagana A, Salerni E, Barluzzi C, Dedominicis V (1999). Mycofloristic investigations in the geothermal area of Travale – Radicondoli (Tuscany, Central Italy). Webbia 54:149-173.

Phillips R (2013). Mushrooms: A comprehensive guide to mushroom identification, Pan Macmillan.

Pidlich-Aigner H, Hausknecht A, Scheuer C (2001). Annotated list of macromycetes found in the greenhouses of the Botanic Garden of the Institute of Botany in Graz (Austria), 1998-2001. Fritschiana 32:49-61.

Razaq A, Shahzad S (2015) . New record species of family Lintinaceae from Pakistan. FUUAST J. Biol. 5(1):17-19.

Redhead S (1997). Standardized Inventory Methodologies for Components of British Columbia's Biodiversity: Macrofungi. Resource Inventory Committee, Vancouver.

Ryvarden L, Gilbertson RL (1993). European polypores. Part 1. Synopsis Fungorum. 6:1-387.

Saba E (1991). Jordan wild mushrooms; their ecology, distribution, classification and toxicity. Thesis, Jordan University, Amman (Jordan). Department of Biological Sciences.

Senthilarasu G (2015). The lentinoid fungi (Lentinus and Panus) from Western ghats, India. IMA fungus 6(1):119-128.

Sesli E, Denchev CM (2008). Checklists of the myxomycetes, larger ascomycetes, and larger basidiomycetes in Turkey. Mycotaxon 106:65-67.

Sharma SK, Atri NS (2015). The genus Lentinus (Basidiomycetes) from India - an annotated checklist. J. Threat. Taxa 7:7843-7848.

Solak M, Alli H, Işiloğlu M, Kalmiş E (2009). Some new records of Inocybe (Fr.) Fr. from Turkey. Turk. J. Bot. 33:65-69.

Vašutová M (2006). Preliminary checklist of the genus Psathyrella in the Czech Republic and Slovakia. Czech Mycol. 58(1-2):1-29.

Venturella G (2006). 2006. Pleurotus nebrodensis. The IUCN Red List of Threatened Species. 2006:e.T61597A12506882.

Venturella G, Zervakis G, Raimondo F (2002). Mycology in sustainable development: The case of Pleurotus nebrodensis from Sicily (Southern Italy). J. (Anon.). Protocols, A Guide to Methods and Applications.

Watanabe T (2010). Pictorial atlas of soil and seed fungi: morphologies of cultured fungi and key to species. CRC Press.

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Full Length Research Paper

Nondestructive maturity assessment tools for commercially viable fruits and vegetables in Uganda

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Fruit and vegetable maturity at harvest influences transportation and storage requirements as well as market value. However, small scale farmers in Uganda lack technologies for maturity assessment leading to high pre- and post-harvest losses and low financial returns from fruit and vegetable farms. This study, therefore, assessed the development of fruits (pineapple, passion fruit, watermelon) and vegetables (cabbage, egg plant, pumpkin and tomato), determined optimal maturity indices and fabricated and tested nondestructive tools for maturity assessment. Propagation trials and testing of tools were undertaken at Nangabo and Kangulumira sub counties in central Uganda. The findings show that eggplant, passion fruit, pineapple and pumpkin underwent 3 distinct development stages during which their diameter, length and outer colour changed. Cabbage remained green, but its bulb diameter and length varied with maturity. Watermelon had 4 development stages with significant (P≤0.05) changes in morphology and outer colour. Tomato fruits had 6 distinct outer colour changes. Age and colour were maturity indices for passion fruit. Fruit age and diameter were the maturity indices for watermelon. Age, diameter, length and colour were the maturity indices for pineapple, tomato and eggplant. Cabbage and pumpkin share indices including: age, diameter and length. A farm record book (FRB) was designed for documenting phenology and maturity stages of studied fruits and vegetables. A calibrated calliper (CC) and Calibrated tape (CT) were fabricated for assessing the morphological development in pineapple, tomato, eggplant, cabbage, pumpkin and watermelon. Customized colour charts (CCC) were designed for monitoring colour changes as passion fruits, pineapples, tomatoes and eggplants mature. On-farm trials show that CCCs were the most effective tools for monitoring passion fruit (80%), pineapple (64%), tomato (60%), eggplant (68%). Similar studies involving several cultivars and maturity determination tools are, therefore, recommended.

Key words: Fruit, Kangulumira, maturity index, maturity determination tools, Nangabo, vegetable.

INTRODUCTION

Fruits and vegetables should be harvested with precision to ensure that their maturity meets or exceeds the minimum level acceptable to the consumer at the time they are consumed (Reid, 2002). According to Okiror et al. (2017b), development and use of non-destructive tools for monitoring the maturity of fruit and vegetable is a critical intervention towards determining optimal harvest time and minimizing pre-and postharvest losses. This is so because the maturity level at harvest is vital to the development of good flavor and taste quality in the fruit when fully ripe (Dadzie and Orchard, 1997). Xudong et al. (2009) assert that fruit and vegetable maturity influences

market value, transportation and storage requirements. Thus, it is important for individuals harvesting fruit to have effective methods of determining maturity (Kader and Mitcham, 2008).

Several methods have been developed for the nondestructive determination of fruit and vegetable maturity (Slaughter, 2009). The nondestructive technologies are based on aroma, colour, defects, shape, size, firmness, composition and density (Chen, 1996; Abbott, 1999; Butz et al., 2005). Other destructive tools including laboratory determination of total soluble solids, total titrable acidity, starch content, moisture content and protein content were designed and evaluated for commercial manufacturers (Slaughter, 2009; AOAC, 2000).

The existing methods are expensive and technically robust and thus unapplicable to small-scale Ugandan farmers in the determination of the optimal time of fruit and vegetable harvest (Kato, 2011; Muzaale, 2014). This could be one of the key factors for increasing postharvest losses and food insecurity in Uganda (IPC, 2017). It is, therefore, critical to work with farmers to develop farmer low-cost and technologically adoptable maturity assessment tools that can minimize postharvest losses while at the same time ensuring financially sustainable returns for growers (Hanrahan and Röder, 2017).

This study was an attempt to fill a gap from previous scholars including Kader and Mitcham (2008), Slaughter (2009) and Zhang and McCarthy (2012) who focused on high cost technologies such as near infrared and magnetic resonance imaging, colorimeters and starch testers that may not be affordable to small scale farmers in Uganda. Hanrahan and Röder (2017), assert that for a technology to be useful for any operation, associated instruments should be accurate and reliable, especially when operated under field conditions by personnel with minimum training such as peasants in Uganda. In addition to the initial purchase price, the cost of labor (training, performing of task and maintenance) should be modest (Hanrahan and Röder, 2017).The specific objectives of this study were therefore to (i) investigate the development of fruits (pineapple, passion fruit and watermelon) and vegetables (cabbage, eggplant, pumpkin and tomato), (ii) determine the optimal maturity indices, and (iii) fabricate and test nondestructive tools for fruit and vegetable physical maturity assessment using low-skill and low-cost techniques in Uganda.

MATERIALS AND METHODS

Description of study area

Study fruits (pineapple, passion fruit and watermelon) and vegetables (cabbage, eggplant, pumpkin and tomato) were propagated to determine maturity indices and design nondestructive maturity assessment tools, in Makerere University Agricultural Research Institute Kabanyolo (MUARIK), Nangabo Sub County, Wakiso district, central Uganda (Figure 1). MUARIK is located on coordinates 0°27'60''N, 32°36'24''E and at an altitudinal range of 1,250 to 1,320 m above mean sea level (Yost and Eswaran, 1990).

Komutunga and Musiitwa (2001) reported Kabanyolo to be part of the Lake Victoria basin that receives an average annual precipitation of 1,218 mm and slightly drier periods in June to July and December to February of the year. The average annual temperature is 21.5°C. Kabanyolo soils are formed on residuum and colluvium from quartzites, gneiss and basement complex rocks. A recent study by Okiror et al. (2017a) reported Kabanyolo soils to be acidic, with the pH ranging from 6.08-6.2, and deficient in organic matter and most essential minerals. The texture of MUARIK soil is predominantly clayey. On the side slopes, colluvium enriched with lateritic gravel is common (Yost and Eswaran, 1990). Training and research in agricultural sciences are the main activities conducted at MUARIK by the Makerere University College of Agricultural and Environmental Sciences and collaborating institutions (Okiror et al., 2017a). However, as part of the Buganda surface, the predominant farming system around MUARIK is the banana-coffee system (Kisamba-Mugerwa, 2001).

On-farm testing of nondestructive maturity assessment tools was carried out with fruit and vegetable famers in Kangulumira Sub County, Kayunga district (Figure 1). Kagulumira is located on 0°34'54''N, 33°1'46''E within 1,070 m above mean sea level. Kangulumira sub county is part of Kayunga district that experiences a bimodal rainfall pattern with peaks between March and May and September to December of the year (NEMA, 2016). The rainfall is evenly distributed within the district. Subsistence agriculture employs almost 96% of the population (UBOS, 2016). The major food crops include bananas, sweet potatoes, cassava, maize, beans and ground nuts. Coffee is the main cash crop, but due to the coffee wilt disease, its production is declining. The farmers have opted for fruits (papaya, pineapples, watermelon, mangoes and passion fruit) and vegetables (amaranth, cabbages, eggplants, pumpkins, tomato) to diversify their sources of income and enhance household food security (UBOS, 2016).

Determination of maturity indices

The propagation trials were run between May and August 2015 at MUARIK. Save for pineapple that was propagated using suckers, other fruits (passion fruit and watermelon) and vegetables (cabbage, eggplant, pumpkin and tomato) seedlings were produced following procedures described by Pinho et al. (2011). The soils were ploughed and loosened using hand hoes. Manual watering was done on days in which the site did receive rains as done by most small-scale farmers in Uganda (Kato, 2011). Seedlings were transplanted manually using hand hoes into pre-made holes in 3 randomized blocks on 26 May 2015. As is the case with most local small scale farmers (Muzaale, 2014), study fruits and vegetables were established in the field without application of any fertilization at transplanting. The cultivars that were studied are presented in Table 1.

Plants were tagged and data on leafing, root collar diameter, plant height, pest and disease incidence and weeding and pruning were recorded until flower emergence at 8 weeks (watermelon), 10 and pumpkin) and 15 weeks after transplanting tomato. After flower

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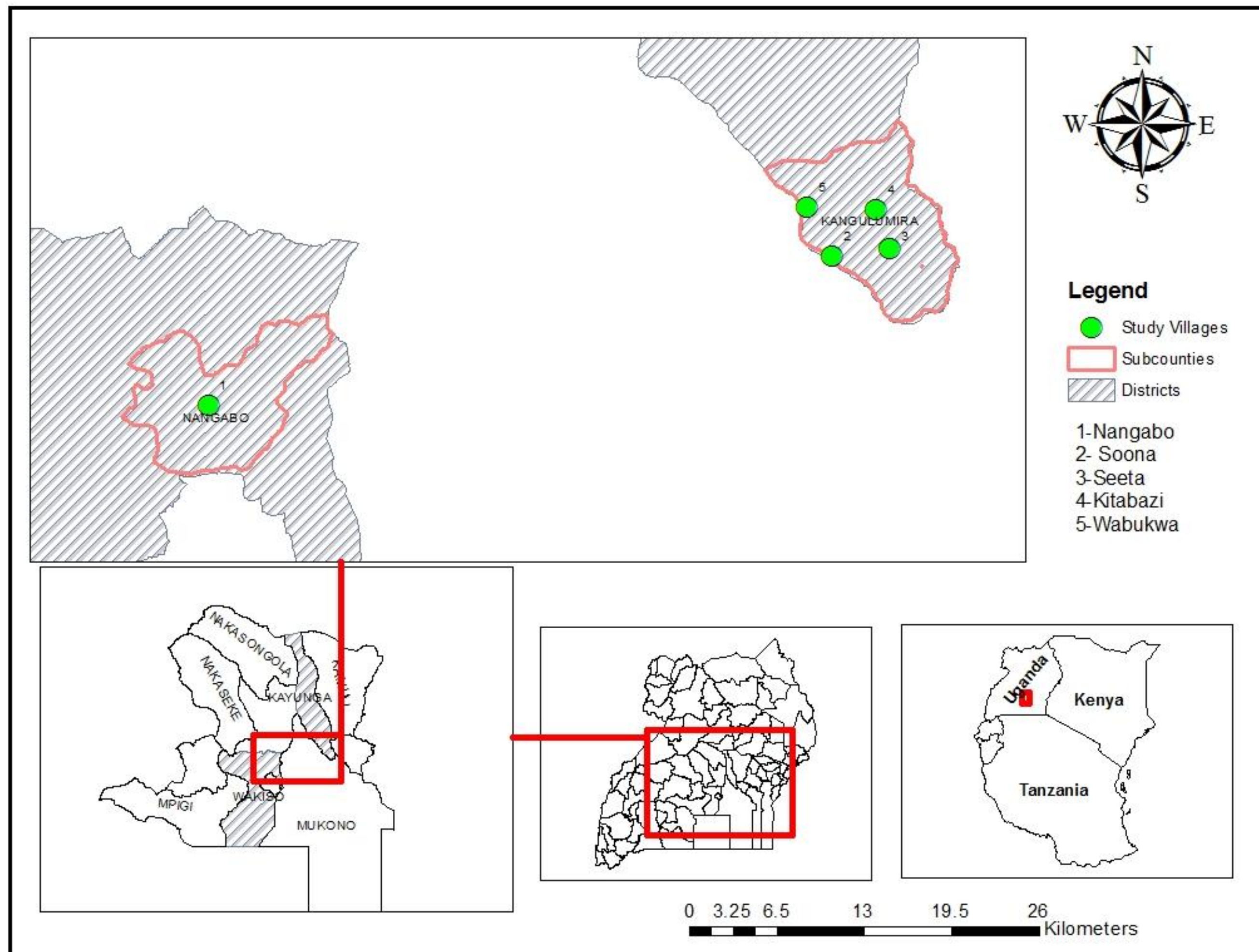

Figure 1. Map of Uganda showing the location of the study sites.

Table 1. Study fruit and vegetable cultivars.

Fruit/vegetable	Experimental code	Scientific name	Family	Cultivar	Seed source
Fruit					
Passion fruit	PSF	*Passiflora edulis* Sims	Passifloraceae	Possum Purple	His Grace Agrochemicals (U)
Watermelon	WTM	*Citrullus lanatus* (Thub.)	Cucurbitaceae	Sugar Baby	Balton (U)
Pineapple	PNP	*Ananus comosus* (L.) Merr.	Bromeliaceae	*NK	Kangulumira, Kayunga district
Vegetable					
Tomato	TMT	*Solanum lycopersicum* L.	Solanaceae	Ghalia 281	Nsanja (U)
Eggplant	EGP	*Solanum melongena* L.	Solanaceae	Back Beauty	East African Seeds Limited
Cabbage	CAB	*Brassica oleracea* L.	Brassicaceae	Copenhagen Market Cabbage	East African Seeds Limited
Pumpkin	PMK	*Cucurbita pepo* L.	Cucurbitaceae	NK	Mecron Ent. (U)

*NK=Not known.

Table 2. Fruit and vegetable development[1].

Fruit/vegetable	Parameter	Unit	Maturity stage[2]					
			1	2	3	4	5	6
Fruit								
PSF[3]	Diameter	(cm)	4.77±0.24	4.35±0.24	4.20±0.24			
	Length	(cm)	5.10±0.24	4.63±0.24	4.55±0.24			
	Fruit age	(wks)	1.00±1.25	3.00±1.25	4.00±1.25			
	Observed colour	(USDA, 1991)	Green	Turning	Purple			
WTM	Diameter	(cm)	3.50±4.91	9.00±4.91	13.30±4.91	16.60±4.91		
	Length	(cm)	5.60±7.80	14.30±7.80	21.20±7.80	26.40±7.80		
	Fruit age	(wks)	1.00±1.48	3.00±1.48	4.00±1.48	5.00±1.48		
	Observed colour	(USDA, 1991)	Green	Light green	Yellowish green	Light yellow		
PNP	Diameter	(cm)	6.50±0.25	6.80±0.25	7.10±0.25			
	Length	(cm)	6.80±0.42	7.10±0.42	7.80±0.42			
	Fruit age	(wks)	1.00±2.06	4.00±2.06	6.00±2.06			
	Observed colour	(USDA, 1991)	purple	Light purple	Yellow			
Vegetable								
TMT	Diameter	(cm)	4.35±0.16	4.30±0.16	4.70±0.16	4.65±0.16	4.40±0.16	4.35±0.16
	Length	(cm)	5.30±0.19	5.55±0.19	5.15±0.19	5.15±0.19	4.95±0.19	5.10±0.19
	Veg. age	(wks)	1.00±1.34	3.00±1.34	4.00±1.34	4.43±1.34	4.72±1.34	4.86±1.34
	Observed colour	(USDA, 1991)	Green	Breaker	Turning	Pink	Light red	Red
EGP	Diameter	(cm)	7.85±1.63	11.10±1.63	7.45±1.63			
	Length	(cm)	10.15±2.48	12.30±2.48	6.30±2.48			
	Veg. age	(wks)	1.00±1.25	3.00±1.25	4.00±1.25			
	Observed colour	(USDA, 1991)	Purple	Bronze	Dark purple			
CAB	Diameter	(cm)	6.30±1.73	8.80±1.73	10.50±1.73			
	Length	(cm)	6.15±2.05	9.30±2.05	11.10±2.05			
	Veg. Age	(wks)	1.00±1.82	2.00±1.82	3.00±1.82			
	Observed colour	(USDA, 1991)	Green	Green	Green			
PMK	Diameter	(cm)	14.50±0.56	15.85±0.56	15.00±0.56			
	Length	(cm)	8.05±3.78	17.25±3.78	13.00±3.78			
	Veg. Age	(wks)	1.00±1.25	3.00±1.25	4.00±1.25			
	Observed colour	(USDA, 1991)	Green	Turning	Yellow			

[1]Data means±standard deviation; [2]The distinct maturity stages observed in this study were; 6(TMT), 4(WTM) and 3 for PSF, PNP, EGP, CAB and PMK. Beyond these stages, the study fruits and vegetables began to ripen and/or go bad. [3]PSF=Passion fruit, WTM=Watermelon, PNP=Pineapple, TMT=Tomato, EGP=Eggplant, CAB=Cabbage, PMK=Pumpkin.

emergence, fruit development was monitored as described by Pinho et al. (2011). On each study plant, randomly selectedfruits were tagged and fruit diameter, height, color and pest and disease incidence were recorded daily. Similar data was collected from cabbage samples.

Two (2) randomly selected fruits and vegetables were harvested from each of the respective maturity stages (Table 2) but in consideration of the three (3) slope positions in the three (3) propagation blocks, labelled and packed in a cool box (Marina 24S) to avoid manual contact. The harvest sampling of passion fruit (N=54), pineapple (N=54), watermelon (N=72), eggplant (N=54), pumpkin (N=54) and tomato (N=108) was based on outer colour, while cabbage (N=54) selection depended on the size of the bulb. Samples were harvested in the morning based on among other characteristics; (i) uniformity of color, (ii) size and (iii) absence of disease and injury (Pinho et al., 2011). The samples were labeled, packed in a cool box (Marina 24S) and delivered to Makerere University Food Science and Technology Laboratory for physico-chemical and nutritional analysis following Okia et al. (2013). The laboratory tests were undertaken to determine the morphological indicators that correlate with chemical parameters such as total soluble solids, protein content, total titrable acidity, pH and carbohydrate content.

Maturity assessment tools

Nondestructive and noninvasive tools were fabricated based on maturity indices for study fruits and vegetables. Local Artisans and Fine Artists in Nangabo Sub County were engaged to develop Farm Record Books (FRB), Calibrated Callipers (CC), Calibrated Tapes (CT) and Customized Colour Charts (CCC). The calibrations were based on fruit and vegetable development and maturation data (diameter, height, age and colour) and physico-chemical and

nutritional analyses results on total soluble solids, carbohydrate content, total titrable acidity, pH and protein content. FRBs were designed to enhance farmers' capacity to document and archive the planting or sowing dates, leafing, flowering, fruiting, maturity, quantity and quality of fruits and vegetables (Utegi and Utegi, 2014). While the CCs and CTs were fabricated using locally cut steel for assessing the morphological (diameter and length) development of pineapple, tomato, eggplant, cabbage and pumpkin (Muchui et al., 2010). Customized colour charts (CCC) were designed for monitoring colour changes in passion fruit, pineapple, tomato and eggplant (Dadzie and Orchard, 1997). Tools were calibrated using the fruit and vegetable planting or sowing dates, leafing, flowering, fruiting, diameters, lengths and colours. Thus, FRBs, CCCs, CTs and CTs were developed for each study fruit and vegetable.

On-farm validation of maturity assessment tools

To validate the non-destructive maturity assessment tools, on-farm tests were undertaken with 20 fruit and vegetable farmers in 4 villages in Kangulumira Sub County, Kayunga district in December 2016. Kangulumira sub county was selected because it is among the reknown areas for fruit and vegetable production in Uganda (Kato, 2011). The farmers were randomly selected from Kitabazi, Seeta, Soona and Wabukwa villages based on their Local Leaders' lists as done by Agea (2010). Each farmer was tasked to use the tool and report whether it was effective or non-effective in determining fruit and/or vegetable maturity. Priority ranking was based on the number of farmers that cosidered the tool effective. A similar approach was used for farmer-led priority ranking in eastern and northern Uganda (Okia, 2010). The tools that were subjected to on-farm validation included FRB, CC, CT and CCC.

Data analysis

Data obtained from the monitoring of fruit and vegetable growth, physico-chemical and nutritional laboratory tests and on-farm validation of maturity assessment tools were entered in MS Excel (Vers. 2010) computer software. The Pearson's correlations (r) were computed to determine the associations between morphological (age, diameter, length and colour) and nutritional maturity indices (total soluble solids, carbohydrate content, total titrable acidity, pH and protein content). Using MS Excel (Vers. 2010), analysis of variance (ANOVA) was run at 5% significance level to ascertain the most significant maturity indicators that were subsequently used to design the maturity assessment tools. Microsoft excel was used to generate frequencies and percentages that guided the priorititzation and ranking of maturity assessment tools.

RESULTS AND DISCUSSION

Fruit and vegetable development

From the propagation experiment, three distinct development stages were observed for passion fruit during which its morphology (diameter and length) and outer colour changed (Table 2). The highest diameter (4.77 cm) was observed at green stage, followed by turning (4.35 cm) and the least was at purple passion fruits (4.20 cm). Fruit length also decreased with maturity of passion fruits, from 5.10 cm at green stage, 4.63 (turning) to 4.55 cm in purple samples. Watermelon had four (4) development stages with significant changes in the diameter, length and outer colour of the fruit. Fruit diameter and length increased as the fruit matured. The highest diameter of 16.60 cm was observed in the 4th stage (light yellow), followed by 13.30 cm in the 3rd stage (yellowish green) and the least (3.50 cm) was recorded in the 1st stage of fruit development. Fruit length increased from 5.60 cm at green stage, 21.20 (yellowish green) to 26.40 cm in light yellow watermelons (Table 2). The morphological changes in a watermelon are similar to what was observed in pineapple samples. Fruit diameter increased from 6.5 (purple), 6.8 (light purple) to 7.1 cm to yellow stage, while fruit length changed from 6.8, 7.1 to 7.8 in the purple, light purple and yellow pineapples, respectively.

Tomato fruit had six (6) highly ordered development stages during which its morphology (diameter and length) and outer colour changed (Table 2). The peak diameter (4.70 cm) was at turning, followed by 4.65 cm at pink stage and the lowest diameter (4.30 cm) was in the breaker. Fruit length was highest (5.55 cm) in the breaker, followed by green (5.30 cm) and lowest (4.95 cm) in the light red stage. Eggplant changed through three stages including purple (diameter =7.85 cm, length = 10.15 cm), bronze (diameter = 11.10 cm, length = 12.30 cm) to dark purple (diameter = 7.45 cm, length = 6.30 cm). Cabbage bulbs remained green through three distinct morphological changes. At stage 1, diameter was 6.3 cm and length was 6.15 cm. In stage 2, cabbage bulbs were 8.8 cm wide and 9.3 cm long. The 3rd stage of cabbage had an average diameter of 10.5 cm and 11.1 cm long. Pumpkin took three stages to mature including green, turning to yellow in which fruit diameter changed from 14.5 cm, 15.85 cm to 15.0 cm and length increased from 8.05 to 17.25 cm then dropped to 13.00 cm, respectively (Table 2).

Similar observations have been reported in other studies. For example Wu and Kubota (2008), observed tomato fruit enlarge with time after anthesis during the green stage, reach maximum size at around the end of the green stage and hardly change in size after the breaker stage through the red stage. Additionally, the changes in fruit diameter and length usually arise from variations in the cellular structure and internal structure of the fruit (Zhang and McCarthy, 2012). Similarly, this study reveals the unique changes in the diameter and length in the fruits (pineapples, passion fruit and watermelon) and vegetables (cabbage, eggplant, pumpkin and tomato) that were investigated.

In addition, fruit and vegetable maturity correlates with color which usually reflects the biochemical changes during ripening (Zhang and McCarthy, 2012). For example, tomatoes progressed from green to breaker, light pink, light red to red when fully mature. To enhance acceptance of fruit by consumers, tomatoes should be harvested at breaker stage for distant markets and fully

Table 3. Morphological maturity indices.

Fruit/vegetable	Maturity index*			
	Age	Diameter	Length	Colour
Fruit				
PSF[1]	0.04*			0.01**
WTM	0.35*	0.05*		
PNP	0.04*	1E-10**	0.021*	1E-11**
Vegetable				
TMT	1E-10**	0.05**	1E-13**	0.02*
EGP	1E-9**	0.03*	0.43*	1E-07**
CAB	0.02*	0.02*	0.04*	
PMK	0.03*	0.02*	0.04*	

[1]PSF = Passion fruit, WTM = Watermelon, PNP = Pineapple, TMT = Tomato, EGP = Eggplant, CAB = Cabbage, PMK = Pumpkin; Morphological parameters with * and ** were significant maturity indices at 0.05 and 0.01 alpha level, respectively when correlated with nutritional composition (total soluble solids, carbohydrate content, total titrable acidity, pH and protein content) of the samples.

Table 4. Maturity assessment tools.

Fruit/vegetable	Maturity index and measurement tool			
	Age	Diameter	Length	Colour
Fruit				
PSF[1]	*FRB[1]	CC		CCC
WTM	FRB	CC		
PNP	FRB	CC	CT	CCC
Vegetable				
TMT	FRB	CC	CT	CCC
EGP	FRB	CC	CT	CCC
CAB	FRB	CC	CT	
PMK	FRB	CC	CT	

[1]PSF=Passion fruit, WTM=watermelon, PNP=pineapple, TMT=tomato, EGP=eggplant, CAB=cabbage, PMK=pumpkin; FRB=farm record book; CC=calibrated calliper; calibrated tape; CCC=customized colour chart.

ripe for local markets (Dadzie and Orchard, 1997). Other fruit and vegetable colour changes that were monitored in this study are relevant in the planning of optimal harvest regimes (Muchui et al., 2010), especially by small scale farmers in Uganda.

Maturity indices

The maturity indices for study fruits and vegetables are presented in Table 3. Fruit age ($P \le 0.05$) and colour ($P \le 0.05$) were significant for passion fruits. It also emerged that fruit age ($P \le 0.05$) and fruit diameter ($P \le 0.05$) were significant for watermelon. In addition, fruit age ($P \le 0.05$), diameter ($P \le 0.05$), length ($P \le 0.05$) and colour ($P \le 0.05$) were significant for pineapples (Table 3). Tomato maturity can be gauged from the changes in its age ($P \le 0.05$), diameter ($P \le 0.05$), length ($P \le 0.05$) and colour ($P \le 0.05$). Similarly, eggplant development can be monitored based on age ($P \le 0.05$), diameter ($P \le 0.05$), length ($P \le 0.05$) and colour ($P \le 0.05$). Cabbage and pumpkin shared maturity indices including age ($P \le 0.05$), diameter ($P \le 0.05$) and length ($P \le 0.05$) (Table 3).

The revelation of age as a maturity indicator for the sample fruits and vegetables observed in this study (Table 3) is supported by findings from other previous studies. According to Hanrahan and Röder (2017), the time of day and prevailing weather conditions influence product maturity and shelf life of fruits and vegetables. Further more, Mattheis and Fellman (1999) opin that fruits and vegetables harvested at an early stage of maturity are susceptible to shriveling and mechanical damage and develop poor flavor and taste, despite having long storage life. In contrast, harvesting at an advanced stage of maturity produces fruits and vegetables that have good taste and flavour, but have a short storage life and are not suitable for transporting for long distances (Dadzie and Orchard, 1997). This implies that fruit and vegetable age can be monitored as an indicator of maturity.

Colour is a useful indicator of fruit and vegetable maturity despite Zhang and McCarthy (2012) argument that during tomato processing, the fruit fed to the processing line are usually a mixture of tomatoes of multiple cultivars with varying maturity conditions. Thus, as much as colour is a significant index of maturity, it may not be reliable for a mixture of cultivars. Molyneux et al. (2004) stated that fruit or vegetable skin color may vary tween cultivars despite the cultivars falling within the same maturity stage. However, Dadzie and Orchard (1997) support external color because its assessment is noninvasive and nondestructive and does not require high skilled staff.

Morphological parameters such as diameter and length are equally vital maturity indicators. Muchui et al. (2010) demonstrated the relevance of the changes in fruit length and diameter in maturity determination. It was therefore recommended that the use of noninvasive tools be promoted among rural farmers to improve harvesting regimes, minimize postharvest losses and improve income and food security (Muchui et al., 2010; Robinson, 1996).

Maturity assessment tools

Based on the significant maturity indices in Table 3, the following tools were developed to enhance small-holder farmers' capacity to assess fruit and vegetable maturity. A farm record book (FRB) was designed for documenting the planting or sowing dates, leafing, flowering, fruiting, maturity, quantity and quality of fruits and vegetables (Table 4). A calibrated caliper (CC) and calibrated tape(CT) were developed with local artisans for assessing the morphological (diameter and length) development of

pineapple, tomato, eggplant, cabbage and pumpkin. Customized colour charts (CCCs) were designed for monitoring colour changes in passion fruit, pineapple, tomato and eggplant (Table 4).

As shown in Table 2, fruit and vegetable maturity can be determined by the number of weeks after fruit onset. Therefore, farmers with proper records can predict the optimal time of harvest and plan for transportation and marketing of fruit and vegetable products. The FRB should be used concurrently colour charts, callipers and diameter tapes to determine the optimal time of harvest. A farm record book refers to a tool for systematic documentation of all activities and transactions regarding all aspects of farm operations (Batte and Foster, 2008). Okojie and Ayinde (2012), categorized farm records into inventory, production (e.g. phenology, yield and maturity), expenditure and income and special supplementary records. To make FRBs useful, the records must be accurate, neat and complete and should be filled-out as soon as the operation or transaction occurs (Okojie and Ayinde, 2012). Utegi and Utegi (2014) analyzed the activities of a farmer and showed the relevance of farm records and accounting in agricultural production.

As much as FRBs are recommended, their adoption may face challenges including illiteracy and limited campaign by government agencies towards record keeping in Uganda. These challenges could be addressed through (i) issuance of low-interest bank loans to farmers as an incentive for farm record keeping, (ii) illiterate farmers employing relatives who are educated as data clerks, (iii) low taxation charges from government for farmers who keep farm records and (iv) distribution of fertilizers and subsidized farming tools to only farmers who keep records (Utegi and Utegi, 2014). Just like in most African countries, most small scale farmers in Uganda are less-educated, unwilling and financially unable to employ Clerks. Famers undertake mental record-keeping and accounting. There is, therefore, need to encourage documentation through farmer extension services (Winkler, 2008) to enhance farmers' capacity to plan produce harvesting, transportation and marketing.

Calibrated callipers (CC) and calibrated tapes (CT) were developed with local artisans for assessing the morphological (diameter and length) development of pineapple, tomato, eggplant, cabbage, pumpkin and watermelon. Slaughter (2009), recognizes shape and size as characteristics for which nondestructive methods for assessing fruit and vegetable maturity. It is preferable to use noninvasive and nondestructive tools such as CC and CTs that can show structural variations as fruit or vegetable mature (Zhang and McCarthy, 2012). Besides, the CCs and CTs can be fabricated and maintained using locally available steel or woody material by artisans within the farmer community.

Customized colour charts (CCC) were designed for monitoring colour changes in passion fruits, pineapples, tomatoes and eggplants. The CCCs are noninvasive and nondestructive and can be used to assess fruit maturity and vegetable in the field, market or inspection points (Dadzie and Orchard, 1997). In addition, Slaughter et al. (2006) used nondestructive optical measurements in the visible region to measure maturity of peaches. Similarly, the USDA colour classification chart for fresh fruits is one of the most handy tools for assessing maturity and classifying fruits (USDA, 1991). Besides colour charts, other tools have been used to determine maturity from colour. For example, Malevski et al. (1977) used a colorimeter to assess skin colour of fruits. Jha et al. (2006, 2007) used a portable spectrophotometer to measure the average skin color and reflectance spectra of fruits and Ornelas-Paz et al. (2008) nondestructively measured the skin color of mangoes during ripening using a colorimeter. However, Malevski et al. (1977) assert that outer skin color measured with a colorimeter at an arbitrarily selected site on the fruit was an unreliable index of maturity. This study, therefore, recommends the use of low cost and low-tech CCCs as tools for fruit and vegetable maturity assessment by small-scale farmers in Uganda.

On-farm validation of maturity assessment tools

On-farm trials of the maturity tools was undertaken with 20 fruit and vegetable farmers in 4 villages in Kangulumira Sub County, Kayunga district (Table 5). It emerged that calibrated colour charts (CCCs) were the most effective tools for assessing the maturity of passion fruit (80%), followed by farm record books (20%). For watermelon, the farmers appraised calibrated callipers (68%) and farm registers (32%). Pineapple maturity assessment tools that were highly ranked by farmers included calibrated colour charts (64%), farm registers (24%) and calibrated callipers (08%).

Relatedly, calibrated colour charts (60%), farm registers (28%) and calibrated tapes (04%) were preferred for tomato monitoring by the study group. For eggplant assessment, the farmers prioritized calibrated colour charts (68%) and calibrated callipers (20%) and detested farm registers (4%). The tools preferred for monitoring the maturity of cabbage were calibrated callipers (44%), farm record books (32%) and the least was calibrated tapes (24%). Similarly, calibrated callipers (40%), farm record books (36%) and calibrated tapes (24%) were validated for pumpkin maturity measurement (Table 5).

The effectiveness of CCCs reported by farmers is not surprising because external fruit and vegetable appearance can be used to predict the internal characteristics (Slaughter, 2009). In addition, colour charts have been used by the United States Department of Agriculture for rapid maturity assessment for decades (USDA, 1991). The noninvasive and nondestructive nature of colour charts could also be another factor

Table 5. On-farm validation of maturity assessment tools.

Fruit/vegetable	Priority ranking of maturity tools by farmers		
	Name	% effectiveness (N=20)	Rank
Fruit			
PSF[1]	FRB	20	2
	CCC	80	1
WTM	FRB	32	2
	CC	68	1
PNP	FRB	24	2
	CC	08	3
	CT	04	4
	CCC	64	1
Vegetable			
TMT	FRB	28	2
	CC	08	3
	CT	04	4
	CCC	60	1
EGP	FRB	04	4
	CC	20	2
	CT	08	3
	CCC	68	1
CAB	FRB	32	2
	CC	44	1
	CT	24	3
PMK	FRB	36	2
	CC	40	1
	CT	24	3

[1]PSF = Passion fruit, WTM = watermelon, PNP = pineapple, TMT = tomato, EGP = eggplant, CAB = cabbage, PMK = pumpkin; FRB = farm record book; CC = calibrated calliper; calibrated tape; CCC = customized colour chart.

influencing farmers' choice. In addition, the use of CCCs does not require highly skilled personnel and is, therefore, appropriate for illiterate farmers (Dadzie and Orchard, 1997).

Calibrated callipers (CCs) and calibrated diameter tapes (CTs) were validated for cabbage, eggplant, pineapple, pumpkin and tomato maturity measurement (Table 5). The fabricated CCs and CTs measure the outer diameter and length of the sample and are therefore nondestructive to the fruits or vegetable products. According to Zhang and McCarthy (2012) and Dadzie and Orchard (1997), it is advisable for small holder farmers to use easy-to-use and low cost maturity assessment tools. Perhaps the farmers noticed the simplicity and ease of fabrication and maintenance of the CCs and CTs that they prioritized to use them over other tools in their fruit and vegetable farms.

Farm record books were preferred for monitoring all the study fruits and vegetables by the sampled farmers (Table 5). The preference for FRBs is not uncommon among farmers especially literate ones. World over, farm record keeping is a reknown best practice. Other scholars including Batte and Foster (2008), Okojie and Ayinde (2012) and Utegi and Utegi (2014) agree that FRBs should be used to document inputs, practices, outputs and for growth and maturity assessment in fruit and vegetable gardens. Perhaps this could be one of the reasons Winkler (2008) makes emphasis on the integration of farm record management in agricultural extension programmes.

Conclusions

This study demonstrates that passion fruit undergoes three distinct development stages during which its morphology (diameter and length) and outer colour change. Watermelon was observed to undergo four (4) development stages with significant changes in the morphology (diameter and length) and outer colour of the fruit. Pineapple fruit diameter and length increased as its outer colour progressed from purple, light purple to yellow stage. Tomato fruits undergo six (6) distinct development stages during which the morphology (diameter and length) and outer colour change. Eggplant diameter and length also changed through three stages including purple, bronze to dark purple. Cabbage remains green throughout three distinct morphological. Pumpkin takes three colour changes to mature including green, turning to yellow with variations in fruit diameter and length (Table 2).

Fruit age and colour were significant ($P \leq 0.05$) indices for passion fruits. Whereas fruit age and diameter were significant ($P \leq 0.05$) for watermelon. Age, diameter, length and colour are the maturity indices for pineapple, tomato and eggplant. Cabbage and pumpkins share the same maturity indices including: age, diameter and length (Table 3).

Furthermore, study findings indicate that a FRB is handiest tool for recording planting dates, leafing, flowering, fruiting and maturity of fruits and vegetables. A calibrated calliper (CC) and calibrated tape (CT) will be useful to for assessing the morphological (diameter and length) development of pineapple, tomato, eggplant, cabbage and pumpkin. Customized colour charts (CCC) are important for montitoring colour changes in passion fruit, pineapple, tomato and eggplant (Table 4). On-farm trial tests of the maturity tools with farmers showed CCCs as the most effective tools for assessing the maturity of passion fruit (80%), pineapple (64%), tomato (60%), eggplant (68%). 44, 40 and 68% of the farmers preferred CCs for monitoring the maturity of cabbage, pumpkins and watermelons, respectively (Table 5).

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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REFERENCES

Abbott JA (1999). Quality measurement of fruits and vegetables. Postharvest Biol. Technol. 15:207-225.

Agea JG (2010). Use and potential of wild and semi-wild food plants in alleviating household poverty and food insecurity: A case study of Bunyoro-Kitara Kingdom, Uganda. Ph.D Thesis. School of Environment, Natural Resources & Geography, Bangor University, Bangor, U K. 360+xiv pp.

AOAC (2000). Association of Official Analytical Chemists. Official methods of analysis of the AOAC international. 17. ed. Washington, DC: AOAC.

Batte M, Foster L (2008). Ohio Farm Computer Usage. Farm Management Update, Winter 2003-04.20.

Butz P, Hofmann C, Tauscher B (2005). Recent developments in noninvasive techniques for fresh fruit and vegetable internal quality analysis. J. Food Sci. 70(9):R131-R141.

Chen P (1996). Quality evaluation technology for agricultural products. Seoul, Korea: Agricultural Machinery Engineering.In. Proc. Intl. Conf. November 12-15, pp. 171-204.

Dadzie BK, Orchard JE (1997). Routine Post Harvest Screening of Banana/Plantain Hybrids. Criteria and Methods. INIBAP Technical Guidelines 2. IPGRI, Rome.

Hanrahan I, Röder S (2017). New Tools to Help Determine Maturity of Tree Fruit. Washington State University Tree Fruit. TIANNA.DUPONT(http://treefruit.wsu.edu/news/new-tools-to-help-determine-maturity-of-tree-fruit/).

IPC (2017). Report of the integrated food security phase classification Analysis for Uganda. Prepared by Uganda IPC Technical Working Group, January 2017. 80p.

Jha SN, Chopra S, Kingsly ARP (2007). Modeling of color values for nondestructive evaluation of maturity of mango. Food Eng. 78:22-26.

Jha SN, Kingsly ARP, Chopra S (2006). Non-destructive determination of firmness and yellowness of mango during growth and storage using visual spectroscopy. Biosyst. Eng. 94(3):397-402.

Kader A, Mitcham B (2008). Optimum Procedures for Ripening Mangoes. In. Fruit Ripening and Ethylene Management: 47-48. Univ. Calif. Postharvest Technology Research and Information Center Publication Series #9.

Kato J (2011). Green house tomato growers root for market. Agribusiness Magazine, New Vision, January 11th, 2011.

Kisamba-Mugerwa W (2001). Social Background. In: Mukiibi (Ed). Agriculture in Uganda – General information. NARO- Uganda, pp.186-199.

Komutunga ET, Musiitwa F (2001). Climate. In. Mukiibi, J.K, Ed., Agriculture in Uganda , Volume 1: General Information, Fountain Publishers, Kampala, pp. 21-32.

Malevski Y, Gomez-Brito L, Peleg M, Silberg M (1977). External color as maturity index of mango. J. Food Sci. 42:1316-1318.

Molyneux SL, Lister C, Savage GP (2004). An investigation of the antioxidant properties and colour of glasshouse grown tomatoes. Int. J. Food Sci. Nutr. 55:537-545.

Muchui MN, Njoroge CK, Kahangi EM, Onyango CA (2010). Determination of Maturity Indices of Tissue Cultured Bananas (*Musa* spp.) 'Williams' and 'Grande Naine'. Proc IC on Banana & Plantain in Africa. Eds.: T. Dubois et al. Acta Hort. 879, ISHS 2010.

Muzaale F (2014). Growing good quality tomatoes. Farming Magazines, Daily Monitor. Wednesday January 8th, 2014.

NEMA (2016). State of the Environment Report for Uganda 2014. National Environment Management Authority (NEMA), Kampala.

Okia CA (2010). *Balanites aegyptiaca*: A resource for improving nutrition and income of dryland communities in Uganda. Phd thesis, University of Wales, Bangor, United Kingdom. 310 p.

Okia CA, Kwetegyeka J, Okiror P, Kimondo JM, Teklehaimanot Z, Obua J (2013). Physico-Chemical Characteristics and Fatty Acid Profile of Desert Date Kernel Oil. Afr. Crop Sci. J. 21(3):723-734.

Okiror P, Lejju JB, Bahati J, Rugunda GK, Sebuuwufu CI, Mulindwa P, Ocan JJ (2017a). Suitability of Kabanyolo Soils for Fruit and Vegetable Production. Open J. Soil Sci. 7:19-33.

Okiror P, Lejju JB, Bahati J, Rugunda GK, Sebuuwufu CI (2017b). Maturity Indices of Tomato (*Solanum lycopersicum* L.), cv. Ghalia 281 in Central Uganda. Afr. J. Agric. Res. 12(14):1196-1203.

Okojie LO, Ayinde IA (2012). Course materials for AEM 302, Principles of Farm Management. Open Courseware, Unoversity of Agriculture, Abeokuta, Nigeria.

Ornelas-Paz JJ, Yahia EM, Gardea AA (2008). Changes in external and internal color during postharvest ripening of 'Manila' and 'Ataulfo' mango fruit and relationship with carotenoid content determined by liquid chromatography-APcI+-time-of flight mass spectrometry. Postharvest Biol. Technol. 50:145-152.

Pinho L, Almeida AC, Costa CA, Paes MCD, Glória MBA, Souza RM (2011). Nutritional properties of cherry tomatoes harvested at different times and grown in an organic cropping. Hortic. Bras. 29:205-211.

Reid MS (2002). Maturation and maturity indices. In. Postharvest technology of horticultural crops, A. A. Kader, ed. Univ. of California, Oakland, CA, USA: ANR Publication 3311. pp. 55-62.

Robinson JC (1996). Bananas and Plantains. 2nd edition. University Press, Cambridge.

Slaughter DC (2009). Nondestructive Maturity Assessment Methods for Mango: A Review of Literature and Identification of Future Research Needs. Biological and Agricultural Engineering, University of California, Davis, January 2009.

Slaughter DC, Crisosto C (2006). Nondestructive determination of internal quality in clingstone peaches. ASABE Paper 066180. St. Joseph, Mich. USA: ASABE.

UBOS (2016). The 2016 Statistical Abstract. Uganda Bureu of Statistics, Kampala, Uganda.

USDA (1991). United States standards for grades of fresh tomatoes. USDA, Agricultural Market Service, Washington, DC. (http://www.ams.usda.gov/AMSv1.0/getfile?dDocName=STELPRDC5050331).

Utegi M, Utegi EN (2014). The Importance of Farm Records And Accounting In Agricultural Production. Katsina-Ala Multidiscip. J.

Winkler MM (2008). Farm Accounting from the Viewpoint of the Farm Manager. J. ASFMRA 2(1):27-56.

Wu M, Kubota C (2008). Effects of high electrical conductivity of nutrient solution and its application timing on lycopene, chlorophyll and sugar concentrations of hydroponic tomatoes during ripening. Sci. Hortic. 116:122-129.

Xudong S, Hailiang Z, Yande L (2009). Nondestructive assessment of quality of Nanfeng mandarin fruit by a portable near infrared spectroscopy. Int. J. Agric. Biol. Eng. 2(1):65-71.

Yost D, Eswaran H (1990). Major Land Resource Areas of Uganda.World Soil Resources. Soil Conservation Service-USDA. Washington D.C, USA. 227p.

Zhang L, McCarthy MJ (2012). Measurement and evaluation of tomato maturity using magnetic resonance imaging. Postharvest Biol. Technol. 67:37-43.

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Combining ability and heterotic orientation of mid-altitude sub-humid tropical maize inbred lines for grain yield and related traits

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Information on the combining ability and heterotic pattern of elite inbred lines is essential to maximize their use in hybrid maize development. This study was conducted to determine combining ability and heterotic pattern of locally developed maize inbred lines for grain yield and related traits. Seventeen inbred lines (10 female inbred lines and 7 tester inbred lines) were used to generate 70 single cross hybrids using line by tester crossing scheme. The resulting 70 cross progenies plus two standard checks arranged in 8×9 alpha lattice design replicated twice were planted at three mid-altitude sub-humid testing sites in Ethiopia (Bako, Hawassa and Pawe) in 2011 main cropping season. The combined analysis of variance for yield and other related traits showed highly significant differences among genotypes, crosses, female inbred lines (General combining ability, GCA), tester inbred lines (GCA), line x tester (Specific combining ability, SCA); and the interactions of these source of variation with the environment for all traits studied except for ear aspect (EA) and grain yield (GY) in female inbred lines (GCA), EA in inbred line testers (GCA) and for days to anthesis (AD) in line x tester (SCA) x environment. The significance of both GCA (lines and testers) and SCA of LxT for AD, days to silking (DS), plant height (PH), ear height (EH), EA and GY showed that both additive and non-additive gene actions are important in controlling these traits. Furthermore, the proportion of GCA sum of squares were greater than the SCA sum of squares for AD, DS, PH, EH, and EA indicating the predominance of additive gene actions in controlling these traits. For GY, the ratio of GCA to SCA sum of squares was near to unity indicating both additive and non-additive gene actions were equally important. This study identified inbred lines that can make good cross combination for more than one trait. L1 was found to be good combiner for lower values of AD, DS, PH and EH indicating that this line could be used in improving maize for earliness and short stature. L4 was ideal parent for reducing AD and DS. L3 was found to be good combiner for GY and other related traits. In addition, lines were grouped into heterotic group A, B or AB based on SCA. Based on its per se performance and combining ability, L3 was proposed to be used as a tester in heterotic group B. This study also validated T5 remain to be used as a tester in heterotic group A. Based on the SCA of crosses, heterosis and per se performance of the parents, five best cross combinations were identified for possible release or for use as parents of three way hybrids. Further verification of the stability of the selected hybrids and the new proposed tester across more locations needs to be done.

Key words: General combining ability, specific combining ability, heterotic pattern.

INTRODUCTION

Maize (*Zea mays* L.), together with wheat (*Triticum aesivum* L.) and rice (*Oryza sativa* L.) is one of the three most important cereal crops that feed two - third of the world population (Ji et al., 2013). In Sub - Saharan Africa (SSA), maize is a major staple cereal food crop with the significance comparable to rice in South East Asia and wheat in the Middle East. Maize being the highest yielding cereal crop in the world is of significant importance for countries like Ethiopia, where future food supply would be a great challenge for the rapidly increasing population. Ethiopia currently produces more maize than any other crop (McCann, 2005; Abate et al., 2015; FAOSTAT, 2015). In 2014, maize was grown on about 2.1 million hectares, resulting in total annual production of 7.2 million tons, with an average of 3.42 tons per hectare (FAOSTAT, 2015). It is a primary crop in majority of farming systems and staple food of the rural population in much of the mid-altitude sub-humid agro-ecology of the country. Maize stands first in total production and productivity and, second in area coverage (20.27%) next to tef [*Eragrostis tef* (Zucc) Trotter] (30.66%) of all cereal crops cultivated in Ethiopia (FAOSTAT, 2015).

The hybrid development in Ethiopia has been highly effective in increasing maize yields since the commercialization of the hybrids in the country. The national average grain yield increased from about 1.6 ton ha^{-1} in 1990 (Abate et al., 2015; Worku et al., 2002; Mosisa et al., 2011) to 3.42 tons ha^{-1} in 2014 (FAOSTAT, 2015). Increased yields are in part due to improved agronomic practices and increased inputs, but increased yields could not have been realized without genetic improvements (Abate et al., 2015). However, the current national productivity average per hectare (3.42 tons ha^{-1}) (FAOSTAT, 2015), is considerably low as compared to developed countries. The national average grain yield per hectare of some of the countries, for example, is 10.68, 10.73, 9.36, 5.18, and 6.0 tons ha^{-1} respectively for USA, Canada, Germany, Brazil and China (FAOSTAT, 2015). This emphasizes that further genetic improvement efforts have to be made to increase maize productivity in the country.

Information on the combining ability of parental inbred lines is very important for determining breeding strategies, classifying the parental inbred lines, defining heterotic groups, and predicting future hybrid performance (Xingming et al., 2004; Legesse et al., 2009). Classifying inbred lines into heterotic groups is critical to determine the potential usefulness of the inbred lines for the development of high yielding hybrids and synthetic varieties. Therefore, knowledge on the heterotic groups of inbred lines is important before they can be deployed in variety development.

Understanding the genetic basis for hybrid performance and identifying parental inbred lines that form superior hybrids is crucial in designing appropriate breeding strategies. Further advancement in the yield of maize requires certain information regarding the nature of combining ability of the parents available for use in the breeding program as well as the nature of gene action involved in expression of both quantitative and qualitative traits of economic importance.

General combining ability (GCA) and specific combining ability (SCA) effects are important indicators of the potential value of inbred lines in hybrid combinations and in grouping materials into heterotic groups. The use of heterotic groups, when aided with good testers in a breeding program can result in the production of high yielding hybrids. Testers of hybrid value or heterosis between parental inbred lines can increase the efficiency of hybrid breeding programs (Legesse et al., 2009). Estimates of GCA and SCA will help to devise breeding and selection strategies. The use of GCA and SCA estimates has proven effective in maize breeding program to identify superior hybrids, find the best parent for hybrid formation, and choose material for new heterotic groups (Hallauer and Miranda, 1958). Although some genetic studies have been conducted in maize on commercial inbred lines (Dagne, 2002; Dagne et al., 2010; Hadji, 2004), it is a continuous and an endless process that the information on combining ability and heterotic pattern of the newly developed inbred lines needs to be determined. Therefore, the objectives of this study were (a) to determine the combining ability of new mid-altitude sub-humid tropical maize inbred lines (b) to categorize the inbred lines into different heterotic groups based on SCA and heterosis for grain yield and (c) to identify single crosses which have better agronomic traits than the commercial checks.

MATERIALS AND METHODS

Description of experimental sites

The study was conducted at three locations in the mid-altitude sub-humid agro ecology of Ethiopia; namely, Bako, Hawassa and Pawe Agricultural Research Centers in the main cropping season of 2011. Bako Agricultural Research Center (BARC) is located at 9°6'N latitude and 37°09'E longitude, 255 km west of Addis Ababa at an altitude of 1650 m above sea level (*masl*). The soil type at BARC is characterized by reddish brown clay (nitosol) with pH (H_2O) of 6.0 (Yadessa et al., 2005). The site receives an average annual rainfall of 1245 mm and the mean minimum and maximum air

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Table 1. List of parental inbred lines used to generate the single cross hybrids using line x tester mating scheme.

Code	Pedigree	Source*	Parental Type	Heterotic group
L1	DE-78-Z-126-3-2-2-1(g)	BNMRC	Female	Unknown
L2	Gibe-1-158-1-1-1	BNMRC	Female	Unknown
L3	Kuleni -320-2-3-1-1-2-1	BNMRC	Female	Unknown
L4	Pool9A-105-1-1-1-1	BNMRC	Female	Unknown
L5	X1264DW-1-2-1-1-1	BNMRC	Female	Unknown
L6	30H83-7-1-5-1-1-1-1	BNMRC	Female	Unknown
L7	ILOO'E-1-12-4-1-1	BNMRC	Female	Unknown
L8	ILOO'E-1-9-1-1-1-1	BNMRC	Female	Unknown
L9	SZSYNA99F2-7-2-1-1-1-1	BNMRC	Female	Unknown
L10	SZSYNA99F2-2-7-3-1-1	BNMRC	Female	Unknown
T1	SC22	BNMRC	Male	B
T2	124-b(109)	BNMRC	Male	A
T3	CML395	CIMMYT	Male	B
T4	CML202	CIMMYT	Male	B
T5	CML312	CIMMYT	Male	A
T6	CML442	CIMMYT	Male	A
T7	CML197	CIMMYT	Male	A

BNMRC = Bako National Maize Research Center; CIMMYT = International Maize and Wheat Improvement Center.

temperatures are 13.3 and 27.9°C, respectively. Pawe Agricultural Research Center (PARC) is located at 11°12’N latitude and 36°20’E longitude at an elevation of 1150 *masl*. The annual precipitation of the testing site ranges from 1338.7 to 2005.7 mm with a mean of 1603.5 mm. The mean annual maximum and minimum temperature of the area are 38.54 and 16.36°C, respectively. Hawassa Agricultural Research Center (HARC) is situated at 7°08’N latitude and 38°48'E, 275 km south of Addis Ababa at an altitude of 1700 *masl*. The soil at HARC is volcanic in nature. The site receives average annual rainfall of 1110 mm and the mean minimum and maximum air temperatures are 13 and 27.4°C, respectively.

Experimental materials

Seventy single cross hybrids were generated from crosses of 10 white grained locally developed tropical mid-altitude sub-humid maize inbred lines used as females (L) and seven white grained inbred lines (five from CIMMYT and two from the National Program) used as males/testers (T), using line by tester mating scheme at Bako Research center in 2010 (Table 1). Among the male parents, T1 (from heterotic group B) and T2 (from heterotic group A) were locally developed inbred lines by the national maize breeding program having good combining ability. The remaining five male parents (T3, T4, T5, T6 and T7) were also CIMMYT inbred lines having defined heterotic groups.

Experimental design and trial management

In 2011, the 70 F_1 single cross progenies along with two standard hybrid checks (BH540, single cross hybrid and BH543, three-way cross hybrid) were planted at Bako, Hawassa and Pawe. The experiment was planted in an alpha-lattice (8×9) design (Yadessa et al., 2005), with two replicates. Each entry was planted in a two-row plot of 5.1 m long and 0.75 m apart with a distance of 0.3 m between plants within a row.

The trials were hand planted with two seeds per hill, which later were thinned to one seed per hill to get a total plant population of 44,444 per hectare. Fertilizers were applied as Di-ammonium Phosphate (DAP) and urea at the rates, kg ha^{-1} of 100 N and 100 P_2O_5 at Bako, 110 N and 46 P_2O_5 at Hawassa and 75 N and 70 P_2O_5 at Pawe. All recommended rates of P_2O_5 were applied at the time of planting while N was applied in split, half at planting and the remaining half at 35 to 40 days after planting. Pre-emergence herbicide, Primagram-gold 660 SC was applied at the rate of 3 l ha^{-1} at planting to control weeds.

Data collection and analysis

Data were collected on grain yield (GY) (adjusted to 12.5 grain moisture and expressed as tons ha^{-1}), plant (PH) and ear height (EH) (cm), days to 50% anthesis (DA) and silking (DS), and ear aspect (EA) (1-5 scale) on plot basis at each of the three locations.

The data obtained for different traits from field measurements were organized and analyzed using SAS statistical package (SAS, 2009). The significance of each trait in each location, ANOVA for combining ability for individual locations and across locations was determined.

Analysis of variance per location was conducted with the PROC MIXED procedure in (SAS, 2009) where entries were treated as fixed factor; while location, replication and incomplete blocks within replication were considered as random factors. Entry means adjusted for block effects generated from individual location analysis according to lattice design (Cochran and Cox, 1957) were used to perform the combined analysis using the PROC GLM procedure in SAS (2009). Then, the data were subjected the F test and the significance at 5 and 1% probability levels was evaluated.

For traits that showed significant differences among crosses, further analysis was performed following the procedures of Singh and Chaudhary (1979) and Dabholkar (1999) to partition the mean square due to crosses into lines, testers and line by tester(LxT) effects using SAS computer program (SAS, 2009) for individual location and combined across locations. The mean squares for genotypes, crosses, checks and location were tested against the

Table 2. Mean squares due to genotypes and error for grain yield and related traits in 10 × 7 line by tester maize crosses along with two standard checks evaluated at Bako, Hawassa and Pawe.

Trait	Bako		Hawassa		Pawe	
	Genotype (DF=71)	Error (DF=55)	Genotype (DF=71)	Error (DF=55)	Genotype (DF=71)	Error (DF=55)
DA	11.64**	5.24	4.74**	1.39	5.87**	1.56
DS	12.24**	5.18	3.86**	1.46	6.35**	1.48
PH	722.03**	280.59	559.03**	98.74	594.03**	163.75
EH	441.63**	160.91	343.89**	52.47	405.43**	107.65
EH	0.32**	0.12	0.11**	0.05	1.07**	0.4
GY	4.83**	1.65	3.83**	1.18	2.96**	1.14

**=significant at 0.01 probability level, DF=degree of freedom; ^{ns} = non-significant, DA = days to anthesis, DS = days to silking, PH = Plant height, EH = ear height, EA= ear aspect, GY = grain yield.

mean squares for their corresponding interaction with location as error term while their interaction with location were tested against their corresponding pooled error. Since means (over replications) were used for combined analysis of variance, estimates of pooled error mean squares were calculated following the procedure of Dabholkar (1999).

Estimation of combining ability effects

Genotypic means of individual locations were used for the determination of GCA and SCA. The two standard checks were excluded while analyzing combining abilities. The GCA effects of lines (L) and testers (T), the SCA effect of LxT, and their interactions with the environment were determined following the method stated by Kempthorne (1957), assuming the following model.

$$Y = \mu + g_i + g_j + S_{ij} + e_k + (ge)_{ik} + (ge)_{jk} + (se)_{ijk} + d,$$

Where Y_{ijk}= the performance of the hybrid, made with i^{th} female and j^{th} male, in the kth location, μ = the overall mean, g_i = the effect of the ith female, g_j = the effect of the jth male, s_{ij} = the interaction of the ith female with the jth male, e_k = the effect of the kth environment, $(ge)_{ik}$ = the interaction of the g_i and e_k, $(ge)_{jk}$ = the interaction of the g_j and e_k, $(se)_{ijk}$ = the interaction of s_{ij} and e_k.

Significance of GCA and SCA effects were performed computing the standard error for lines, testers and crosses and then tested against t-test by taking the degree of freedom of pooled error mean square (Singh and Chaudhary, 1979; Dabholkar, 1999; Sharma, 2003). The proportional contributions of lines (GCA_L), testers (GCA_T), and their interaction (SCA_{LxT}) to the sum square of crosses were calculated as the ratio between sum of squares of each component and the cross sum of squares as given by Sharma (2003) and Dabholkar (1999).

Orientation of lines into heterotic group A and B depended on the direction of the specific combining ability such that lines exhibiting positive SCA with tester A were allocated to the opposite heterotic group B, and vice versa, whereas lines displaying positive SCA to both were designated as AB group.

RESULTS AND DISCUSSION

Analysis of variance of Individual location

The mean squares of analysis of variance for different characters at Bako, Hawassa and Pawe are presented in Table 2. At Bako, significant differences were observed among the genotypes in grain yield and other traits (viz. DA, DS, PH, EH, EA). Likewise, the genotypes showed significant differences in their reaction to different foliar diseases such as grey leaf spot (GLS) and Turcicum leaf blight (TLB) (data not shown). Similarly, significant differences in DA, DS, PH, EH, EA, and grain yield among the genotypes were observed at Hawassa. The genotypes were also significantly different in their reaction to common leaf rust (CLR) at this site (data not shown). At Pawe, significant differences were observed among the genotypes in DA, DS, PH, EH, EA, and grain yield. There were no foliar diseases observed in the experimental field during the cropping season at Pawe.

While there were significant differences among the genotypes in six of the traits under investigation (viz., DA, DS, PH, EH, EA and GY) at each of the three locations, there were either no significant differences among the genotypes or there were significant differences among the genotypes at most at two environments in the remaining traits under study (data not shown). The combined analysis of variance across environments and combining ability analysis were, therefore, performed only for the traits that were significantly different among the genotypes at each of the three environments.

Combined analysis of variance

The combined analysis of variance for grain yield and other related traits showed highly significant differences (P<0.01) among genotypes, crosses, lines (L), testers (T), L x T; and the interactions of these source of variation with the location for all traits studied except for ear aspect and grain yield in lines, ear aspect in testers and for days to anthesis in line x tester x environment (Table 3). Similarly, significant differences were observed in checks x location and crosses vs check x location for grain yield. The result of the combined analysis of variance across the three environments revealed that

Table 3. Combined ANOVA of combining ability for grain yield and other agronomic traits in 10 × 7 line by tester crosses evaluated at three environments in 2011 main cropping season.

Source of variation	DF	Mean square					
		DA	DS	PH	EH	EA	GY(ton ha^{-1})
Location	2	4660.13**	4462.56**	14124.61**	12644.23**	18.39**	263.7**
Genotypes	71	9.19**	8.86**	961.62**	579.21**	0.44**	3.63**
Cross	69	9.38**	9.03**	976.55**	586.02**	0.44**	3.69**
GCA(L)	9	36.24**	30.01**	4993.41**	2665.78**	0.68*	9.07ns
GCA(T)	6	23.57*	19.02**	1408.67**	1270.7**	1.99^{ns}	7.97**
SCA(LxT)	54	3.32**	4.42**	259.06**	163.31**	0.23**	2.31**
Check	1	5.04^{ns}	6.00^{ns}	337.50^{ns}	16.67^{ns}	0.38^{ns}	2.52ns
Cross vs. check	1	0.22^{ns}	0.14^{ns}	555.62^{ns}	672.03^{ns}	0.13^{ns}	0.69^{ns}
Genotype x Loc.	142	2.23**	2.73**	137.72**	81.09**	0.2**	1.64**
Cross x Loc.	138	2.25**	2.78**	137.88**	80.44**	0.2**	1.64**
GCA (L) x Loc.	18	4.59**	6.11**	159.34*	109.15**	0.22**	4.04**
GCA (T) x Loc.	12	5.08**	3.88**	175.79*	108.39*	0.92**	1.23**
SCA (LxT) x Loc.	108	1.55^{ns}	2.11**	130.09*	72.55*	0.12**	1.29**
Check x Loc.	2	0.54^{ns}	0.50^{ns}	78.00^{ns}	129.17^{ns}	0.03^{ns}	0.9*
Cross vs. check x Loc.	2	2.33^{ns}	1.25^{ns}	186.40^{ns}	77.75^{ns}	0.01^{ns}	2.36**
Pooled error crosses	159	1.36	1.38	90.15	53.2	0.03	0.48
Pooled error genotypes	165	1.36	1.35	90.51	53.5	0.03	0.47
Pooled error checks	3	1.21	0.67	67.5	75.5	0.02	0.09
GCA (L)(%)		50.4	43.35	66.7	59.33	20.04	32.07
GCA (T)(%)		21.85	18.32	12.54	18.86	39.25	18.77
SCA (%)		27.72	38.32	20.76	21.81	41.1	49.05

*Significant at 0.05 probability level; **significant at 0.01 probability level; DF = degree of freedom; DA = days to anthesis; DS = days to silking; PH = Plant height; EH = ear height; EA = ear aspect; GY = grain yield.

there were significant differences among the genotypes for days to anthesis days to silking, plant height, ear height, ear aspect and grain yield. Various scientists (Asefa, 2004; Zeleke and Nepir, 2007; Kanyamasoro et al., 2012) have also reported similar findings in other groups of inbred lines they studied.

The significance of the mean squares of GCA (of lines and testers) and SCA of L x T for the traits under investigation indicated the importance of both additive and non-additive gene effects for these traits. This is consistent with the findings of other authors (Meseka et al., 2006; Alam et al., 2008; Wegary et al., 2011). The combined ANOVA of combining ability showed that the GCA sum of squares of lines were greater than the GCA sum of squares of testers for all traits except ear aspect. Similarly, the GCA/SCA ratio being greater than unity for days to AD, DS, PH and EH, indicated that additive gene effects were more important for these traits (Table 3) in this group of inbred lines. For grain yield, the GCA to SCA sum of square ratio being slightly near to unity indicated that both additive and non-additive genetic effects were equally important in the inheritance of this trait. Significant mean squares (P<0.01) of environments for DA, DS, PH, EH, EA and GY revealed that the responses of the genotypes across the three environments were different for these traits. The significance of mean squares due to genotypes (P<0.01) for the traits studied indicated the existence of genetic variability among the genotypes.

The significance of the interaction of GCA of parents (lines and testers) with the environment and SCA of the crosses with the environment revealed that the GCA effects of the parents and SCA of the crosses over the test environments were different.

Mean values of six traits (*viz*. DA, DS, PH, EH, EA and GY) averaged across three environments for top 10 entries and GY advantage over the best check are presented in Table 4.

DA ranged from 69.83 to 79.33 days with a mean of 74.94 days. Similarly, DS ranged from 73.00 to 81.67 with a mean of 77.85. The lowest mean values for both DA and DS were observed in cross L4xT2, while the highest mean values for both the traits were observed in the cross L6xT6. As a result, L4xT2 could be preferred for early maturity.

For PH, the crosses ranged from 190.5 to 277.17 cm with a mean of 243.5 cm. Similarly, the crosses ranged from 89.33 to 160.67 cm for EH while the mean was124.90 cm. The highest mean values for PH and EH were observed in the cross L3xT5; while the lowest mean

Table 4. Combined data of top 10 entries based on mean grain yield evaluated at Bako, Hawassa and Pawe Agricultural Research Centers, 2011.

Cross	DA	DS	PH	EH	EA	GY (ton ha^{-1})	GY over the best check (%)
L2xT7	76.33	78.67	246.50	138.83	1.67	10.77	19.8
L9xT7	74.00	76.17	233.33	125.17	1.33	10.67	18.69
L8xT1	75.50	77.83	268.00	133.00	1.50	10.58	17.69
L3xT7	75.83	78.33	267.67	150.50	1.50	10.49	16.69
L3xT5	75.83	78.50	277.17	160.67	1.58	10.48	16.57
L2xT5	75.50	77.67	254.33	137.67	1.75	10.47	16.46
L6xT5	78.00	80.33	273.00	133.00	1.92	10.36	15.24
L6xT7	77.50	79.83	272.00	140.00	1.42	10.36	15.24
L3xT1	74.00	76.67	259.50	138.50	2.17	10.27	14.24
L8xT7	75.50	77.50	257.50	135.50	1.42	10.22	13.68
BH540	73.83	77.00	260.50	133.67	2.17	8.99	
BH543	75.67	79.00	245.50	137.00	1.67	7.70	
Entry mean	74.94	77.85	243.51	124.9	2.06	8.68	
Cross mean	74.94	77.85	243.24	124.6	2.06	8.69	
CV%	2.2	2.11	5.53	8.28	21.1	13.26	
LSD	1.88	1.87	15.34	11.79	0.5	1.31	
Maximum	79.33	81.67	277.17	160.67	2.92	10.77	
Minimum	69.83	73	195.5	89.33	1.33	5.74	

DA = days to anthesis; DS = days to silking; PH = Plant height; EH = ear height; EA = ear aspect; GY = grain yield.

values for the same traits (not included in Table 4) were noted for the cross L10xT6 making this cross more preferable for tolerance to lodging. The entries ranged from 1.33 to 2.92 for their EA with a mean of 2.06. The highest and lowest mean values for ear aspect were observed in crosses L4xT1 and L9xT5 (not included in Table 4), respectively. Because the smaller values of EA are desirable, the cross L9xT5 was preferred for this trait. In GY, the entries ranged from 5.74 to 10.77 ton ha^{-1} with a mean of 8.68 ton ha^{-1}. The two checks used in this experiment, BH543 and BH540, had mean grain yield values of 7.70 and 8.99 ton ha^{-1}, respectively. The Mean grain yield of ten entries exceeded the mean grain yield of the best check (BH540 in this experiment) by over 10% is presented in Table 4. Further testing of these hybrids is needed to see their yield stability across more environments.

General combining ability of female inbred lines

The GCA effects of the 10 female inbred lines for GY and related traits are presented in Table 5. Among the 10 inbred lines, only L3 was with significantly positive mean GCA effects for GY. Therefore, this inbred line is good combiner for improving GY. In contrast, L5 had significantly negative GCA effects for GY, indicating, that this inbred line was not good combiner within this group of inbred lines and may not be exploited for GY improvement. In line with the current study, several authors reported either positive or negative significant GCA effects of inbred lines for GY in other group of inbred lines (Legesse et al., 2009; Dagne, 2002; Zeleke and Nepir, 2007; Ahmad and Saleem, 2003; Worku et al., 2009; Mosa, 2010). In contrary to this, Asefa et al. (2008) found non-significant GCA effects for GY.

The results displayed that L2, L6, L7 and L8 had significantly positive GCA effects for DA, whereas L1, L4 and L9 had significantly negative GCA effects for this trait. The remaining three of the 10 female inbred lines had no significant GCA effects for DA. For DS, L6 and L7 had significant positive GCA effects, whereas L1, L3, L4 and L9 had significantly negative GCA effects, and the remaining four of the 10 female inbred lines had positive but non - significant GCA effects. It has been noticeable that the frequency and distribution of rain, and length of the rainy season particularly in the mid altitude sub-humid agro-ecology of Ethiopia where it used to be for more than five consecutive months is becoming less than four months (data not shown) as the past few years were good examples. This is believed to be attributed to the global climate change. This phenomenon, therefore, emphasizes the importance of early to mid-maturing varieties than late maturing varieties in the mid altitude sub-humid agro-ecology of Ethiopia. As a result, due to the importance of relatively early maturing and lower values of DA and DS, inbred lines which had significantly negative GCA effects were considered as good combiners for the improvement of these traits. These results are in general agreement with the findings of many authors (Dagne, 2002; Ahmad and Saleem, 2003; Mosa, 2010; Bello and Olaoye, 2009).

Table 5. Estimates GCA effects of female inbred lines for grain yield and related traits combined across three environments.

Lines	DA	DS	PH	EH	EA	GY(ton ha^{-1})
DE-78-Z-126-3-2-2-1(g)	-4.43**	-4.16**	-60.18**	-22.61**	0.09^{ns}	-1.81 ^{ns}
Gibe-1-158-1-1-1	2.47*	1.42^{ns}	7.87**	20.21**	-0.08^{ns}	1.88^{ns}
Kuleni -320-2-3-1-1-2-1	-1.72^{ns}	-2.48*	50.41**	61.84**	-0.05^{ns}	3.45**
Pool9A-105-1-1-1-1	-6.11**	-5.27**	23.63**	19.65**	0.79^{ns}	-1.85^{ns}
X1264DW-1-2-1-1-1	0.03^{ns}	0.86^{ns}	11.29**	-18.42**	0.48^{ns}	-2.11*
30H83-7-1-5-1-1-1-1	6.09**	5.12**	61.08**	22.44**	0.41^{ns}	0.26^{ns}
ILOO'E-1-12-4-1-1	4.07**	4.7**	7.18**	-10.12**	0.02^{ns}	-1.69^{ns}
ILOO'E-1-9-1-1-1-1	2.75**	1.63^{ns}	6.97**	5.49**	-0.5^{ns}	1.37^{ns}
SZSYNA99F2-7-2-1-1-1-1	-2.27*	-2.27*	-24.48**	-21.7**	-1.09^{ns}	1.19^{ns}
SZSYNA99F2-2-7-3-1-1	-0.88^{ns}	0.45^{ns}	-83.75**	-56.77**	-0.08^{ns}	-0.71^{ns}
SE (Lines)	0.34	0.34	0.34	0.34	0.34	0.34
SEd (Lines)	0.44	0.44	3.59	2.76	0.06	0.26

*=significant at 0.05 probability level, **=significant at 0.01 probability level; DA = days to anthesis; DS = days to silking; PH = Plant height; EH = ear height; EA= ear aspect; GY = grain yield.

Table 6. Estimates of GCA effects male tester inbred lines for grain yield (GY) and related traits combined across three environments.

Testers	DA	DS	PH	EH	EA	GY(ton ha^{-1})
SC22	0.37^{ns}	0.48^{ns}	11.99**	0.61ns	0.59^{ns}	-0.28^{ns}
124-b(109)	-6.79**	-4.65**	-3.14**	-16.62**	0.22^{ns}	-1.62^{ns}
CML395	-0.85^{ns}	-1.95^{ns}	-16.31**	1.55 ^{ns}	0.28^{ns}	-2.47*
CML202	-0.24^{ns}	-1.34^{ns}	11.59**	6.01**	-0.29^{ns}	-0.92^{ns}
CML312	4.96**	5.35**	32.73**	20.26**	-0.69^{ns}	2.25*
CML442	0.16^{ns}	-0.4^{ns}	-52.51**	-47.01**	1.6^{ns}	-0.34^{ns}
CML197	2.39*	2.51*	15.64**	35.19**	-1.71^{ns}	3.37**
SE (testers)	0.25	0.25	0.25	0.25	0.25	0.25
SEd (testers)	0.37	0.37	3	2.31	0.05	0.22

* = significant at 0.05 probability level; ** = significant at 0.01 probability level; DA = days to anthesis; DS = days to silking; PH = Plant height; EH = ear height; EA = ear aspect; GY = grain yield.

Seven of the 10 female inbred lines (*viz*., L2, L3, L4, L5, L6, L7 and L8) had significantly positive GCA effects for PH while the remaining three lines (L1, L9 and L10) had significantly negative GCA effects for the same trait (Table 5). While five of the 10 inbred lines (*viz*. L2, L3, L4, L6 and L8) had significantly positive GCA effects for EH, the remaining five inbred lines (*viz*. L1, L5, L7, L9 and L10) had significantly negative GCA effects for this trait. Again, due to the reason that the lower plant height and ear height, the more the plant is expected to be resistant / tolerant to lodging, inbred lines which had significantly negative GCA for these traits were considered to be desirable for the improvement of PH and EH. Similar findings have been reported by several authors (Hadji, 2004; Mosa, 2010; Rahman et al., 2010).

General combining ability of tester inbred lines

The GCA effects of the seven tester inbred lines for GY and other five agronomic traits are presented in Table 6. Two of the seven male inbred lines, namely; T5 and T7 had significantly positive GCA effects for GY, whereas T3 had significantly negative GCA effects for the same trait. Consequently, T5 and T7 were considered good combiners for the improvement of GY within this group of inbred lines included in this study. The GCA effects of the remaining four tester inbred lines were not significant for grain yield, all with the same negative sign with different magnitudes.

The GCA effects of two of the seven male tester inbred lines (*viz*. T5 and T7) were significantly positive for both DA and DS while that of T2 was significantly negative for these traits. Therefore, T2 is preferable for the improvement of these traits. The GCA effects of the remaining four of the seven tester inbred lines for both AD and DS were not significant either positively or negatively. For PH, four tester inbred lines namely; T1, T4, T5 and T7 had significantly positive GCA effects whereas T2, T3 and T6 had significantly negative GCA

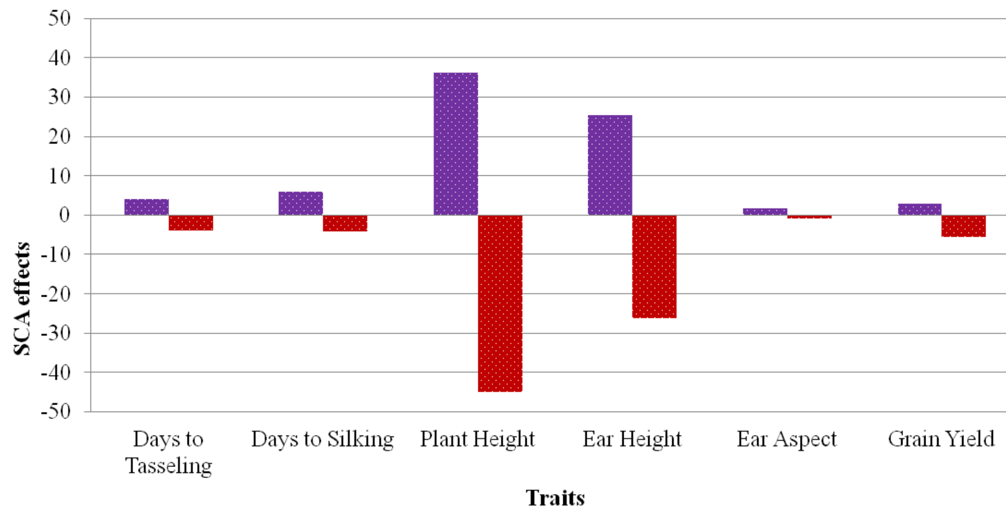

Figure 1. Maximum and minimum SCA effects of crosses for grain yield and other five related traits combined across three locations.

effects. Three of the seven tester inbred lines (*viz*. T4, T5, and T7) had significantly positive GCA effects for EH, while two tester inbred lines (*viz*. T2 and T6) had significantly negative GCA effects for the trait. The GCA effect of none of the tester inbred lines was significant for EA (Table 6).

Specific combining ability of crosses

Maximum and minimum estimates of specific combining ability effects (SCA) of the 70 crosses across three locations for GY and other five related traits are presented in Figure 1. Two of the 10 female inbred lines (L1 and L8) had significantly positive SCA effects when crossed to T1 for GY implying that there was significant positive interaction of genes between the two parents for this trait. Such gene interactions lead to the expression of heterosis, which can be exploited in the development of hybrid varieties. Similarly, the crosses of L4xT3 and L5xT2 had significantly positive SCA effects for GY indicating the importance of non-additive gene action in these cross combinations (data not shown).

For AD, the crosses L1xT7, L4xT4, L5xT5, L5xT6, L6x T6, L7xT2, L7xT3, L9xT1 and L10x T3 had significantly positive SCA effects, and, hence, were not good cross combinations for this trait, as early maturing varieties are desired. Conversely, the crosses L1 x T6, L2 x T5, L3 x T2, L4 x T2, L5x T2, L5 x T3, L6 x T1 and L6 xT4 had significantly negative SCA effects for AD indicating that these crosses were good cross combinations for early maturity. The crosses L1xT7, L4xT4, L4xT7, L5x T5, L5xT6, L6xT6, L7xT3, L8xT5, L10xT2 had significant positive SCA effects for DS indicating that these cross combinations were not desirable crosses for early maturity. The crosses L1xT6, L2xT5, L4xT2, L4xT6, L5x T2, L5xT3, L7x T7, L8xT7, L9x T7 and L10 x T6 had significant negative SCA effects for DS. These crosses were considered good cross combinations for early maturity (data not shown).

Fifty-eight of the 70 crosses had significant SCA effects for PH, and out of that 30 were with positively significant and 28 were negatively significant SCA effects (data not shown). The crosses L1xT7, L4xT4, L8xT3, L3xT2 and L9x T1 were with the highest significant negative SCA effects for PH and were considered the best cross combination for being likely to be tolerant to lodging. Likewise, 31 of the 70 crosses had significant negative SCA effects for EH indicating that these cross combinations may be exploited for tolerance to lodging. None of the crosses had significant SCA effects for EA. Crosses L1xT7 and L8xT1had the highest positive and negative SCA effects for EH, respectively. The crosses that had negative values of SCA for EA were considered desirable as the higher values of EA score indicates bad ear characteristics.

Heterotic orientation

Grain yields of 70 crosses averaged over three environments and estimates of SCA effects of these crosses are presented in Table 7. Among the male inbred lines, T1 and T2 are locally developed inbred lines by the national maize breeding program having good combining ability; and have been used as testers in maize breeding for mid-altitude sub-humid agro-ecology of Ethiopia. Either one or both of these inbred lines are present in pedigrees of two of the commercial hybrids used as standard checks in this study (T1 in pedigree of BH540, and both T1 and T2 in pedigree of BH543). SC22 was developed from SC5522 (heterotic group B) while

Table 7. Heterotic orientation of 10 female lines using SCA estimates with five CIMMYT tester lines with defined heterotic groups.

Females lines	CML395		CML202		CML312		CML442		CML197		GCA of lines	Heterotic orientation
	HGB		HGB		HGA		HGA		HGA			
	SCA	GY	SCA	GY	SCA	GY	SCA	GY	SCA	GY		
DE-78-Z-126-3-2-2-1(g)	-0.11^{ns}	7.40	1.29^{ns}	8.56	0.27^{ns}	8.78	-0.19^{ns}	7.88	-5.69**	5.74	-1.81^{ns}	A
Gibe-1-158-1-1-1	-0.2^{ns}	8.61	-1.3^{ns}	8.38	1.04^{ns}	10.47	0.25^{ns}	9.39	1.09^{ns}	10.77	1.88^{ns}	B
Kuleni -320-2-3-1-1-2-1	0.85^{ns}	9.73	0.17^{ns}	9.74	0.11^{ns}	10.48	-0.27^{ns}	9.64	-0.38^{ns}	10.49	3.45**	B
Pool9A-105-1-1-1-1	2.46*	8.81	-0.47^{ns}	7.57	-0.96^{ns}	8.08	0.28^{ns}	8.13	0.92^{ns}	9.40	-1.85^{ns}	A
X1264DW-1-2-1-1-1	0.72^{ns}	7.76	-1.59^{ns}	6.86	-0.65^{ns}	8.16	-1.02^{ns}	7.32	-0.03^{ns}	8.78	-2.11*	B
30H83-7-1-5-1-1-1-1	-1.37^{ns}	7.41	-0.73^{ns}	8.14	1.85^{ns}	10.36	0.05^{ns}	8.72	1.35^{ns}	10.36	0.26^{ns}	B
ILOO'E-1-12-4-1-1	0.01^{ns}	7.51	-0.7^{ns}	7.50	1.59^{ns}	9.55	0.78^{ns}	8.46	1.07^{ns}	9.54	-1.69^{ns}	B
ILOO'E-1-9-1-1-1-1	-3.56**	6.57	1.03^{ns}	9.50	-1.49^{ns}	8.89	0.3^{ns}	9.24	0.41^{ns}	10.22	1.37^{ns}	B
SZSYNA99F2-7-2-1-1-1-1	1.8^{ns}	9.49	1.42^{ns}	9.66	-0.58^{ns}	9.33	-1.5^{ns}	8.18	1.34^{ns}	10.67	1.19^{ns}	AB
SZSYNA99F2-2-7-3-1-1	-0.59^{ns}	7.51	0.87^{ns}	8.70	-1.19^{ns}	8.34	1.33^{ns}	9.10	-0.09^{ns}	9.23	-0.71^{ns}	B
GCA of testers	-2.47*		-0.92^{ns}		2.25*		-0.34^{ns}		3.37**	-0.28^{ns}		

* = significant at 0.05 probability level; ** = significant at 0.01 probability level; HGA = heterotic group A; HGB = heterotic group B; GY = grain yield.

124-b (109) was developed from Kitale Synthetic II (heterotic group A) (Tolesa et al., 1993). The testers T3, T4, T5, T6 and T7 are CIMMYT inbred lines having known heterotic groups with T3 and T4 being in heterotic group 'B' whereas, T5, T6 and T7 are in heterotic group 'A' (Tolesa et al., 1993). Four of these (*viz*. T3, T4, T5 and T6) have been used as testers in CIMMYT and other national and international maize breeding programs in the tropics. The heterotic grouping of the locally developed inbred lines in the present study was, therefore, based on using these CIMMYT established testers. Based on the assumption that SCA and heterosis of two inbred lines from different heterotic groups is greater than those from the same group, the heterotic orientation of the 10 inbred lines studied in this study will be useful for further breeding work in the national maize breeding program.

The inbred line L1 had no significant SCA effects for GY when crossed with T3, T4, T5 and T6, except with T7 where it had significant negative SCA effect (Table 7). This inbred line also gave the lowest GY (5.74 ton ha^{-1}) when crossed to T7. This, therefore, indicated that L1 and T7 were highly likely to be in the same heterotic group. As a result, L1 was assigned to heterotic group A. Although L2 had no significant SCA effects when crossed with testers of both heterotic group A and B, it gave high heterosis when crossed with testers of heterotic group A (Table 7). Consequently, L2 was postulated to be in heterotic group B. L3 was assigned to heterotic group B because it gave relatively higher heterosis when crossed with testers from heterotic group A (10.48 ton ha^{-1} with T5 and 10.49 ton ha^{-1} with T7); and it had no significant SCA effects when crossed with testers from both heterotic groups.

L4 had no significant SCA effects when crossed to T4, T5, T6, and T7. The inbred line L4 when crossed with T3 exhibited the highest significant positive SCA effect (2.46**). This revealed that L4 and T3 could be in different heterotic groups and, thus, L4 was assigned to heterotic group A. The inbred lines L5, L6 and L7 had no significant SCA effects when crossed with testers from both heterotic groups. Nevertheless, due to the higher heterosis observed when L5, L6 and L7 were crossed to heterotic group A than to heterotic group B; these lines may be assigned to heterotic group B, but need further investigation. The inbred line L8 displayed highly significant negative SCA effect when crossed to T3 and showed no significant SCA effects with the rest testers. This indicated that L8 and T3 were in the same heterotic group and, thus, L8 was assigned to heterotic group B. There were no testers whose cross combination with L9 had significant SCA effects. However, all crosses of L9 with testers from both heterotic groups gave high heterosis (ranged from 8.18 tons ha^{-1} to 10.67 tons ha^{-1}). Consequently, L9 was postulated to be in AB heterotic group. Despite the absence of cross combination that had significant SCA effect when crossed with L10, this inbred line had the highest negative SCA value when crossed to T3. In addition, this cross combination had the lowest mean grain yield (7.51 tons ha^{-1}) compared to the cross of L10 with the rest of the testers indicating that these two inbred lines (L10 and T3) might be in the same heterotic group.

Conclusion

This study identified four new inbred lines and one tester inbred line (L1, L3, L4, L9 and T2) that were good combiners for reducing days to anthesis (DA) and day to silking (DS). Owing to the seasonal rainfall distribution and its inadequacy over much of the mid-altitude

sub-humid agro-ecology of Ethiopia, rainfall is the overriding factor determining maize production in the potential maize producing areas of the country. The main rainy season being frequently not exceeding 3 to 4 months imposes limitations on the permissible delay in planting and early cessation before maturity ultimately causing substantial grain yield reductions. The inbred lines having significant negative GCA for DA and DS identified in the present study could, therefore, be used as parents for breeding maize for earliness in the mid–altitude sub-humid maize growing agro-ecology. Also, the three female inbred lines and three male inbred line testers (*viz.* L1, L9, L10, T2, T3 and T6) having significant negative GCA effects for PH and EH identified in the present study can also be used as parents for breeding maize for short stature to reduce the grain yield loss caused by lodging.

Likewise, the study identified 10 best cross combinations (*viz*. L2xT7, L9xT7, L8xT1, L3xT7, L3xT5, L2xT5, L6xT5, L6xT7, L3xT1 and L8xT7) that exceeded the best check (BH540 in this study) by over 10% in mean GY. Out of the 10 cross combinations, five had T7 as a common parent and T7 is a commercial line released as male parent of BH543. This inbred line has been used as a tester in the mid-altitude maize growing agro-ecology of Ethiopia having positive GCA as exemplified in the present study. However, it is no longer used as a tester due to its poor *per se* performance, especially in areas where turcicum leaf blight is important (data not shown). Consequently, the crosses that were selected based on their higher mean grain yield and have T7 as parent in their pedigrees have less value for commercial use. Of the remaining five crosses identified based on their heterosis over the best standard check, three (L3xT5, L2xT5, L6xT5) had T5 as a parent in their pedigrees. The high significant positive GCA effect for GY observed in the present study coupled with the good *per se* performance (data not shown) of this tester (T5), confirms that it can be used as parent in hybrid maize breeding for commercial release or for further breeding activities. Besides, the current study validated that T5 could remain to be used as a tester (in hetrotic group A) in mid- altitude maize breeding program until better tester is identified. Because L2, L3 and L6 were the elite inbred lines in their *per se* performance (data for per se performance not shown for all inbred lies), the crosses L3xT5, L2xT5, L6xT5 were considered the promising single cross hybrids for possible release after further verification of their performance across more environments, or for use as a single cross parents for predicting three-way cross hybrids. The inbred line T3 had the highest significant positive GCA effect for grain yield besides its good line *per se* performance. As a result, this inbred line was proposed to be used as a tester in heterotic group B. The rest two cross combinations (L3xT1 and L8xT1) selected based on their heterosis over the standard check having T1 as a common parent in their pedigrees, were also considered promising because both lines (L3 and L8) and the tester had good *per se* performance. In general, five promising single cross hybrids (L3xT5, L2xT5, L6xT5, T3xT1 and L8xT1) were identified based on their GY and their respective parental inbred lines' *per se* performance.

As a further step, verifying the utility of the proposed new tester (L3) in early generation testing of lines needs to be conducted in order to give more information on the stability of its combining ability, and relate its performance to the previously used testers. In addition, further evaluation of the selected hybrids under more testing locations in order to get more information on Genotype x environment interactions needs to be done. The five single cross hybrids identified based on their GY and parents' *per se* performance in this study, should be used to decide on either to use them for possible release or for predicting the performance of new three-way cross hybrids. The information obtained from this study on the heterotic pattern and combining ability of the inbred lines for different traits would be useful in planning hybrid maize breeding and predicting the hybrid performance.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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REFERENCES

Abate T, Shiferaw B, Menkir A, Wegary D, Kebede Y, Tesfaye K, Kassie M, Bogale G, Tadesse B, Keno T (2015). Factors that transformed maize productivity in Ethiopia. Food Secur. 7(5):965-981.

Ahmad A, Saleem M (2003). Combining ability analysis in Zea mays L. Int. J. Agric. Biol. 5:239-244.

Alam A, Ahmed S, Begum M, Sultan M (2008). Heterosis and combining ability for grain yield and its contributing characters in maize. Bangladesh J. Agric. Res. 33(3):375-379.

Asefa B (2004). Heterosis and combining ability of transitional highland maize (*Zea mays* L.) inbred lines. http://agris.fao.org/agris-search/search.do?recordID=ET2006000080.

Asefa B, Mohammed H, Zelleke H (2008). Combining ability of transitional highland maize inbred lines. East Afr. J. Sci. 2(1):19-24.

Bello O, Olaoye G (2009). Combining ability for maize grain yield and other agronomic characters in a typical southern guinea savanna ecology of Nigeria. Afr. J. Biotechnol. 8(11): 2518-2522.

Cochran WG, Cox GM (1957). Experimental designs. Published by John Wiley and Sons.

Dabholkar A (1999). Elements Of Bio Metrical Genetics (revised And Enlarged Edition). Concept Publishing Company.

Dagne W (2002). Combining ability analysis for traits of agronomic importance in maize (*Zea mays* L.) inbred lines with different levels of resistance to grey leaf spot (Cercospora zea maydis). MSc Thesis, Haramaya University, Ethiopia.

Dagne W, Vivek B, Berhanu T, Koste A, Mosisa W, Legesse W (2010). Combining ability and heterotic relationships between CIMMYT and Ethiopian maize inbred lines. Ethiop. J. Agric. Sci. 20:82-93.

FAOSTAT F (2015). Food and Agriculture Organization of the United Nations Statistics Division. Rome: FAO.

Hadji T (2004). Combining ability analysis for yield and yield-related traits in quality protein maize (QPM) inbred lines. M. Sc. Thesis. School of graduate studies, Alemaya University, Ethiopia.

Hallauer AR, Miranda J (1958). Quantitative genetics in maize breeding. Iowa State University Press, Ames.

Ji Q, Xu X, Wang K (2013). Genetic transformation of major cereal crops. Int. J. Dev. Biol. 57:495-508.

Kanyamasoro MG, Karungi J, Asea G, Gibson P (2012). Determination of the heterotic groups of maize inbred lines and the inheritance of their resistance to the maize weevil. Afr. Crop Sci. J. 20(s1):99-104.

Kempthorne O (1957). An introduction to genetic statistics. John Wiley & Sons, Inc., New York.

Legesse B, Pixley K, Botha A-M (2009). Combining ability and heterotic grouping of highland transition maize inbred lines. Maydica 54:1-9.

McCann J (2005). Maize and grace: Africa's encounter with a New World crop, 1500-2000. Harvard University Press.

Meseka S, Menkir A, Ibrahim A, Ajala S (2006). Genetic analysis of performance of maize inbred lines selected for tolerance to drought under low nitrogen. Maydica 51(3-4):487.

Mosa H (2010). Estimation of combining ability of maize inbred lines using top cross mating design. J. Agric. Res. Kafer El-Sheikh Univ. 36:1-14.

Mosisa W, Legesse W, Berhanu T, Girma D, Girum A, Wende A, Tolera K, Gezahegn B, Dagne W, Solomon A (2011). Status and future direction of maize research and production in Ethiopia. In. Meeting the Challenges of Global Climate Change and Food Security through Innovative Maize Research. P 17.

Rahman H, Arifuddin Z, Shah S, Iqbal M, Khalil I (2010). Evaluation of maize S2 lines in testcross combinations I: flowering and morphological traits. Pak. J. Bot. 42:1619-1627.

SAS (2009). SAS software, version 9.2. SAS Institute Inc. Cary, NC.

Sharma JR (2003). Statistical and biometrical techniques in plant breeding. New Age International (P) Limited.

Singh RK, Chaudhary BD (1979). Biometrical methods in quantitative genetic analysis. New Delhi : Kalyani Publishers.

Tolesa B, Gobezayehu T, Worku M, Desalegne Y, Mulatu K, Bogale G (1993). Genetic improvement of maize in Ethiopia: A review. In: 1. National Maize Workshop of Ethiopia, Addis Abeba (Ethiopia), 5-7 May 1992, 1993. IAR.

Wegary D, Labuschagne M, Vivek B (2011). The Influence of Water Stress on Yield and Related Characteristics in Inbred Quality Protein Maize Lines and Their Hybrid Progeny. Water Stress 199.

Worku M, Abera W, Tadesse B, Wolde L, Wegary D, Azmach G (2009). Performance of variety cross hybrids of maize (Zea mays L.) in the mid-altitude and highland transition areas of Ethiopia. East Afr. J. Sci. 3(1):80-86.

Worku M, Tuna H, Nigussie M, Deressa A, Tanner D, Twumasi-Afriyie S (2002). Maize production trends and research in Ethiopia. Mandefro, N Tanner, DG, Twumasi-Afriyie, S(eds). pp.10-14.

Xingming F, Bihua H, Feng L(2004). Analysis of combining ability and heterotic groups of yellow grain quality protein maize inbreds. In: Integrated Approaches to Higher Maize Productivity in the New Millennium: Proceedings of the Seventh Eastern and Southern Africa Regional Maize Conference, Nairobi, Kenya, 5-11 February 2002, 2004. CIMMYT, P 143.

Yadessa A, Bekere D, Takele T, Emiru N, Degefe A (2005). Effects of Cajanus cajan biomass transfer and inorganic fertilizer on growth and yield of open pollinated maize variety on acidic nitosols of western Oromia, Ethiopia. In. African Crop Science Conference Proceedings,. pp. 1143-1148.

Zeleke H, Nepir G (2007). Heterosis and Combining Ability in Qpm Versions of Early Generation Highland Maize (Zea mays L.) Inbred Lines. Haramaya University.

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Glycosinolate changes in rapeseed varieties in advanced generations

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The aim of this study was to determine the changes in glycosinolate levels that may arise from cross pollination in the advanced generations in rapeseed. With the greenhouse conditions in addition to two different locations, this study was carried out in 3 locations with two varieties and repeated for 3 years. As a result, the changes in glycosinolate levels in the advanced generations were statistically significant. Although, the increase in the amount of gluconinolate appeared in the 2nd and 3rd generations, it remained below 20 µmol/g.

Key words: Rapeseed, outcrossing, glycosinolate, generations, seed.

INTRODUCTION

Oilseed rape (*Brassica napus*) is an important oilseed crop in most countries and is cultivated for food, feed and non-food uses such as biofuels (Delourme et al., 2013). Rapeseed, is in the third place in the world oil seed production, and mustard cover many species that belong to the Brassiceae family. Brassica species are important oil crops and several species are cultivated worldwide. *Brassica juncea, Brassica napus* and *Brassica campestris* are three widely cultivated species (Alan et al., 2014). It is possible to introgress genetic diversity and specific traits into *B. napus* canola from its progenitor species, *B. oleracea* and *B. rapa* (Bennett et al., 2012).

Glucosinolates are secondary metabolites which are sulphur containing are biosynthesized by plant species in the order Brassicales (Zelmer et al., 2013). Change in glucosinolate content is principally under control of procreative developmental stages (Bhushan et al., 2013). Actual levels of total glucosinolates depend on variety and range from as low as 25 and up to 200 µmol/gram of rapeseed meal (Mavromichalis, 2013). The glucosinolates were reduced due to their negative impact on palatability and toxic effects in many livestock species (Canola Council of Canada, 2015). Canola meal must contain less than 30 µmoles of glucosinolates (Parsons et al., 2016). Breeding has been successful in reducing the glucosinolate content in meal (Savary, 2013). Canola is the genetically improved rapeseed that contains less than 30 µmoles per gram glucosinolates of seed dry matter

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after oil extraction (Hashemi and Beiki, 2014).

Rapeseed is a self-pollinated crop; however, it has a 12to 47% outcrossing rate (Williams et al., 1986; Becker et al., 1992). Beckie et al. (2003) identified the gene flow between commercial areas of varieties with different herbicide resistance properties by evaluating the gene flow data of *B. napus*. Many factors affect outcrossing between plants in fields, these are size of lands, plants fertility, environmental conditions and activity of insect pollinators (Biology Document BIO2007-0, 2012). Several insect pollinators visit canola, especially the honeybee which is one of the most efficient pollinator (Sayed and Teilep, 2013; Mahmoud and Shebl, 2014). Therefore, even if rapeseed cultivation is initiated in an area with "00" type (new type) varieties, using the same seed every year may cause cross pollination with the wild species or with other rapeseed varieties previously cultivated or still being cultivated in the region and lead to changes in the glycosinolate levels. Genetic differences were observed among F2 progenies of *B. napus*/*B. campestris* and their parents (Fayyaz et al., 2014). In the developed synthetic varieties, the decrease in the yield is lower than that in the hybrids. Therefore, it is possible for the producer to use it for several generations without changing the seed. In the case of deterioration in the quality of the seed in terms of the increase in glycosinolate as a result of outcrossing, the pulp obtained from the produced rapeseed will not be suitable for animal health.

There are abundant amounts of related species of rapeseed (cabbage and turnip) in the Çarşamba and Bafra Plains which have the potential to produce rapeseed in a wide area. It is important to determine whether the changes in glycosinolate levels formed in rapeseeds cultivated in regions where cake and turnip are abundant as a result of indehiscence or natural cross pollination with close-relative varieties reach a level that will threaten the animal health. This study was carried out to investigate whether there is a change in the amount of glycosinolate in advanced generations of rapeseed varieties produced under natural conditions.

MATERIALS AND METHODS

In this study, French originated Bristol synthetic variety and German originated Licrown synthetic variety were used as the materials.

A three-year research was carried out to achieve the aim of the study. The field phase of this three-year study covers the preparation of the material to be tested. The seed production phase of the study was carried out at three different locations. Two of these locations were at the field, whereas one of them was carried out at the greenhouse. Çarşamba and Bafra districts at Samsun city in Turkey, which have a wide range of rapeseed cultivation areas, were selected as the location of the field studies.

The research area soil in Bafra contains middle calcareous (3.9%), unsalted (0.017%), very high phosphorus (19.00 kg/da), middle clay (60%) and organic matter is less (1.70%). The Bafra district has a semi-arid climate with annual average minimum and maximum temperatures of 10.3 and 18.3°C, relative humidity of 74.8% and annual rainfall of 737.4 mm.

The research area soil in Çarşamba has middle clay (44%), mild alkalinity (pH 7.88), medium calcareous (10.50%) and salt-free (0.43%). Phosphorus content is high (16.35 kg/da) and organic matter is low (1.71%). The average annual total precipitation, temperature and relative humidity are 648.7 kg, 15.5°C and 74.4%, respectively

Both locations were carefully selected, ensuring that they contain abundant amounts of *Brassica* species (*B. oleraceae* and *B. rapa*) in the close vicinity. The third was the greenhouse application in order to prevent pollen dust from coming from outside. It was investigated whether the possible exchange of pollens between the rapeseeds planted here would have an effect on the changes in glycosinolate levels. The seeds were planted with four replications. The area of each plot was (5x2) 10 m^2. However, plot size was determined as 5 m² under greenhouse conditions. Row spacing was 20 cm and each plot consisted of 10 rows.

At the end of three years (three generations), glycosinolate levels were determined in 72 samples taken from 3 different locations, 2 varieties and 4 replications using NIRS device. The obtained results were subjected to analysis of variance according to randomized complete block experimental design (Gomez and Gomez, 1984).

RESULTS AND DISCUSSION

Glycosinolate levels were determined in rapeseeds produced in different generations varying between 11.90 and 18.88 µmole/g. Although, the differences were statistically not significant, glycosinolate levels in rapeseeds produced under greenhouse conditions were lower than those produced under Bafra and Samsun conditions. This suggests that a small amount of pollen from the environment might be mixed. This result is in line with those stated by Williams et al. (1986) and Biology Document BIO2007-0 (2012).

In the examinations in terms of generations, it was found that 14.03 µmole/g glycosinolate level in the 1st generation had a statistically significant increase in the 2nd generation and reached 15.96 µmole/g. However, the increase ceased in the next generations and determined as 15.86 µmole/g.

Licrown variety (14.49 mmole/g) had a lower glycosinolate content as compared to that of the Bristol variety (16.08 mmole/g) This difference was also statistically significant ($P<0.05$) (Table 1).

The glycosinolate levels in the seeds of Licrown and Bristol varieties increased parallel to each other in the second generation. However, in the next generation, glycosinolate level slightly decreased in Licrown variety, whereas increasing trend continued in Bristol variety (Figure 1). This difference in the trends was possibly due to the different rates of outcrossing in the varieties.

Glycosinolate ratios were found to be acceptable when Licrown and Bristol varieties were left to open pollination for three years. According to Stewart (2002), the pollination time of *Brassica* species is close to that of related species, which poses a risk of pollination with each other. However, it is clear that the probability of natural hybridization of *B. napus* (2n = 36 or 38) due to *B. rapa* (2n = 20), *B. oleraceae* (2n = 18) of different species and chromosome numbers is very low.

Table 1. The changes in erucic acid levels in rapeseeds produced in Central Black Sea Region which might be formed as a result of outcrossing with each other or the close-relative species in the flora*.

Generations	Varieties	Locations			Average
		Samsun	Bafra	Greenhouse	
1st	Bristol	14.82	13.57	14.32	14.24
	Licrown	15.03	14.56	11.90	13.83
	Average	14.92	14.07	13.11	14.03[b]
2nd	Bristol	16.77	16.13	17.28	16.73
	Licrown	14.86	15.88	14.87	15.20
	Average	15.82	16.00	16.07	15.96[a]
3rd	Bristol	17.42	18.42	16.00	17.28
	Licrown	12.84	18.88	14.59	14.44
	Average	15.13	17.15	15.29	15.86[a]
Average of locations		15.29	15.74	14.82	
Bristol					16.08[a]
Licrown					14.49[b]
$LSD_{0.05\ for\ generations}$ =1.60					

*The difference between the averages indicated by the same letter is statistically insignificant within the group.

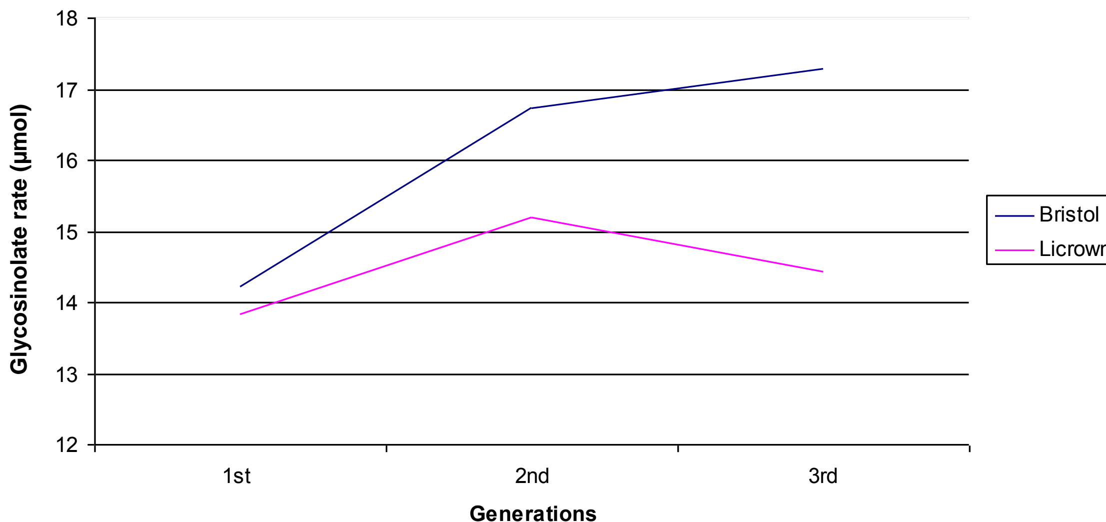

Figure 1. The effect of generation x species interactions on glycosinolate levels.

Conclusion

This study was carried out based on the fact that synthetic varieties have been used unchanged for a couple of generations by some farmers. Glycosinolate levels in the seeds in the advanced generations showed a slight increase; however, did not reach a level in the 3rd generation that will threaten the animal health. Therefore, these synthetic varieties can be used for 2 to 3 years. However, the necessity of changing the seed every year in modern agriculture should not be overlooked.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

REFERENCES

Alan MM, Nahar K, Hasanuzaman M, Fujita M (2014). Alleviation of

osmotic stress in Brassica napus, B. campestris, and B. juncea by ascorbic acid application. Biol. Plant. 58(4):697-708.

Becker HC, Karle R, Han SS (1992). Environmental variation for outcrossing rates in rapeseed (Brassica napus). Theor. Appl. Genet. 84:303-306.

Beckie HJ, Warwick SI, Nair H, Séguin-Swartz G (2003). Gene flow in commercial fields of herbicide-resistant canola (*Brassica napus*). Ecol. Appl. 13:1276-1294.

Bennett RA, Seguin-Swartz G, Rahman H (2012). Broadening Genetic Diversity in Canola Using the C-Genome Species *Brassica oleracea* L. Crop Sci. 52:2030-2039.

Bhushan G, Mishra VK, Iquebal M A Singh YP (2013). Effect of genotypes, reproductive developmental stages, and environments on glucosinolates content in rapeseed mustard. Asian J. Plant Sci. Res. 3(1):75-82.

Biology Document BIO2007-01 (2012). The Biology of *Brassica juncea* (Canola/Mustard). A companion document to the Directive 94-08 (Dir94-08), Assessment Criteria for Determining Environmental Safety of Plants with Novel Traits. http://www.inspection.gc.ca/plants/plants-with-novel-traits/applicants/directive-94-08/biology-documents/brassica-juncea/eng/1330727837568/1330727899677

Canola Council of Canada (2015). Canola Meal Feeding Guide. 5th Edition. Canola Council of Canada, Winnipeg, MB. http://www.canolacouncil.org/media/516716/2015_canola_meal_feed_industry_guide.pdf

Delourme R, Falentin C, Fomeju BF, Boillot M, Lassalle G, André I, Duarte J, Gauthier V, Lucante N, Marty A, Pauchon M (2013). High-density SNP-based genetic map development and linkage disequilibrium assessment in *Brassica napus* L. BMC Genomics 14(1):120.

Gomez AG, Gomez AA (1984). Statistical Procedures for Agricultural Research. 2nd Edition. A Wiley-Interscience Publication, Singapore.

Hashemi SM, Beiki M (2014). The effect of canola meal processing by heat, moisture and ammonium bicarbonate on metabolisable energy and nitrogen retention in broiler chicken. J. Anim. Poult. Sci. 3(4):110-116.

Fayyaz L, Rabbani F, Iqbal MA, Kanwal SM, Nawaz I (2014). Genetic Diversity Analysis of *Brassica napus/Brassica campestris* Progenies Using Microsatellite Markers. Pak. J. Bot. 46:779-787.

Mahmoud MF, Shebl M (2014). Insect fauna of canola and phenology of the diamondback moth, *Plutella xylostella* L. (Lepidoptera: Plutellidae) as A key pest. Redia 97:125-132.

Mavromichalis I (2013). Rapeseed meal in pig, poultry feeds, Geraadpleegd op 12 April 2013 via. http://www.wattagnet.com/articles/14938-rapeseed-meal-in-pig-poultry-feeds.

Parsons CE, Kelly J, Bacon R, Slaton N, Lorenz G, Kring T, Cartwright R (2016). Canola Production in Arkansas. http://www.uaex.edu/publications/pdf/FSA-2154.pdf

Savary R (2013). Evaluating Canola (*Brassica napus*) Meal and Juncea (*Brassica juncea*) Meal With or Without Supplemental Enzymes for Two Commercial Strains of Laying Hens. Submitted in partial fulfilment of the requirements for the degree of Master of Science. Dalhousie University Halifax, Nova Scotia March 2013.

Sayed AMM, Teilep WMA (2013). Role of natural enemies, climatic factors and performance genotypes on regulating pests and establishment of canola in Egypt. J. Basic Appl. Zool. 66:18-26.

Stewart AV (2002). A review of Brassica species, cross-pollination and implications for pureseed production in New Zealand. Agron. New Zealand. 32:63-81.

Williams IH, Martin AP, White RP (1986). The pollination requirements of oil-seed rape (*Brassica napus*). J. Agric. Sci. 106(1):27-30.

Zelmer CD, McVetty PBE, Asif M, Goyal A (2012). Molecular Genetics of Glucosinolate Biosynthesis in Brassicas: Genetic Manipulation and Application Aspects, Crop Plant, Dr Aakash Goyal (Ed.), ISBN: 978-953-51-0527-5, Chapter 9.

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Full Length Research Paper

Phytotoxicity of extracts of *Myrciaria dubia* (Kunth) McVaugh bioprocessed in vegetable crop sensitive to allelochemicals

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The present work is a contribution to the advancement of the scientific knowledge of vegetal species of the Amazon and training of professionals in the area of vegetal and related sciences in the use of alternative methods and techniques more coherent with the context of the socioeconomic and environmental reality of the region. This work aimed to evaluate the effect of extracts prepared from bioprocessed remaining (BR), seeds of fruit of *Myrciaria dubia* (caçari) from the northern Amazon, applied to biological assay with *Lycopersicon lycopersicum* (tomato) seeds. To obtain and classify the degree of phytotoxicity were utilized samples of control and different aqueous extracts, with and without treatment, assessing their physical and chemical quality via pH analysis, electrical conductivity and total of dissolved salts. Thereafter, monitoring of bioassay was held for five days in closed glass plate, with samples incubation at 25 ± 1°C in a dark environment. It was obtained preliminary knowledge about four extracts prepared from T1 and T2 in proportion to 1:10 and 1:100. The methodological process applied allowed the quick examination, up to 120 h, of the effect of the extracts on seed germination and root growth of tomato. Of the four extracts evaluated, those who were previously treated T2D1 and T2D2, regardless of the applied concentration, showed no phytotoxicity to the plant tested and classified according to Germination Index (GI) obtained, 90.2 and 96.6%. The results show an important information about the influence of treatment of the bioprocessed compounds on their chemical and physicochemical characteristics, which can alter their effects on the development of other plants. Therefore, there is possibility of availability of finished products and raw materials from the *M. dubia* seeds, since previously sterilized.

Key words: Caçari, germination index, methodological process, bioprocessed remaining.

INTRODUCTION

The *Myrciaria dubia* is a fruit species that originated from the Amazon, and has been the focus of different

technical-scientific studies in Brazil currently, having prospects of improvement in its production. In this context, in the course of researches, a gap was verifiably completed, referring to the possibility of development of new products after processing of the fruit, known as caçari, in the extreme north of Brazil, making good use only of the pulp and discarding peel and seeds as residues, general practice, widely applied in the northern Amazon yet.

In the processing of the fruits of *M. dubia* for each ton is estimated up to 270 kg, only seeds, which are not yet well exploited, in the Amazon, mainly as a product, possibly due to ignorance or lack of adequate disclosure about its potential, as well as its quality and level toxicity when bioprocessed. It is essential that interdisciplinary projects be developed aimed at increasing the knowledge about vegetal species of the Brazilian biodiversity in the chemical - and toxic-pharmacological interfaces (Campos et al., 2016).

Considering the current methods of toxicological evaluation, in vitro tests offer several advantages, low cost; a small amount of material required; a limited amount of toxic waste, cells and human tissues used; as well as transgenic cells carrying human genes and reduced animal testing (Araujo et al., 2014). According to Araujo and Monteiro (2005), seeds germination and plant growth bioassays are the most common techniques used to assess the phytotoxic compounds According to Trautmann and Krasny (1997), it enables the determination of whether there are, in the compound or in the raw material, substances which may inhibit the germination of seeds, the root growth or development of plants.

Simoneto and Cruz-Silva (2010) reported that the use of seeds of cultivated species and good quality is advisable, including the tomato, easily found and quite sensitive to many allelochemicals (chemical compounds), secondary metabolic products of plants according to Ribeiro et al. (2012).

Researches have shown that certain plants, such as alfalfa, contain water soluble phytotoxic compounds which are released into the soil environment using fresh leaves, stems and crown tissues, as well as dry material, roots in decomposition and seeds (Hall and Henderlong, 1989; Dias de Almeida et al., 2008). In fact, they are according to Weir et al. (2004), who emphasized that allelopathic substances released by the plant, can affect the growth, damage the normal development and even inhibit the germination of seeds of other plant species.

According to Souza Filho et al. (2010), the allelochemicals are found in different parts of the plant including leaves, flowers, roots, stalks, fruits, peels, seeds, and pollen grains. These secondary metabolites may be released directly into the environment by root exudation, volatilization, leaching or decomposition of plant material (Moreno, 1989; Cipollini et al., 2012).

Furthermore, plant secondary metabolites can act in the recipient plant by altering the structure of cell membranes, including the receptors and also there are present flags, capable of causing interference in the cell cycle, modify the action of several hormones, alter the conformation of enzymes and process of transcription and translation (Habermann et al., 2015).

Previous studies demonstrated the phytotoxic potential of aqueous leaf extracts of *Blepharocalyx salicifolius* on the early development of bio-indicator species such as onions, tomatoes and lettuce (Mairesse et al., 2007; Imatomi et al., 2013). In a study conducted by Sausen et al. (2009) the aqueous leaf extracts of *Eugenia involucrata* (Myrtaceae) and *Acca sellowiana* (Myrtaceae) were phytotoxic to the germination and growth of tomato and onion seedlings growth.

However, work on the phytotoxicity of *M. dubia* (Myrtaceae) on these bioindicators using extracts obtained from seeds, remnants of the pulping of fruits of this species were not found in literature.

The vegetables germination test is a widely used model to assess the potential of the plant extracts (allelochemicals or isolated compounds). One of the purposes set out when certain compounds interfere with cell function is the change in the germination rate of seeds, revealing their toxic and/or cytotoxic action (Luz et al., 2012).

However, according to Faria et al. (2009), further studies are needed regarding the forms of extraction, types of extractors, extraction time and application rates, in addition to part of the plants to be used as low phytotoxic effect may occur by low concentrations of compounds inhibitors present in the extracts tested.

According to Noldin et al. (2003), the use of vegetable biological assays to monitor bioactivity of extracts, fractions and compounds isolated from plants is one of the alternatives that have often been incorporated into the identification and monitoring of potentially toxic substances.

In this study, evaluation of the effect of extracts with agrifood potential made from the bioprocessed remaining seeds of the fruit of *M. dubia* was purposively taken from the northern Amazon, applied to biological assay with seeds of *Lycopersicon lycopersicum* (L.) H. Karst. (tomato) in the laboratory to obtain and classify the degree of phytotoxicity with a view to providing raw materials and new process methodological/biotech product for Brazilian society.

This study is a contribution to the advancement of the scientific knowledge of vegetal species of the Amazon

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Table 1. Results obtained on visual inspection absence (-) or presence (+) of microorganisms in different dosages of BR sand (S), sandy soil (SS) and clay soil (CS).

Treatments	Identification	Visual inspection	
		3rd day	7th day
1	100% S	-	-
2	75% S + 25% BR	+	+
3	50% S + 50% BR	+	+
4	25% S + 75% BR	+	+
5	100% SS	-	-
6	75% SS + 25% BR	+	+
7	50% SS + 25% BR	+	+
8	25% SS + 75% BR	+	+
9	100% CS	-	-
10	75% CS+ 25% BR	+	+
11	50% CS + 50% BR	+	+
12	25% CS + 75% BR	+	+
13	100% BR	+	+

and training of professionals in the area of vegetal and related sciences in the use of alternative methods and techniques more coherent with the context of the socioeconomic and environmental reality of the region.

MATERIALS AND METHODS

Study site and provenance of the plant material

The study was conducted at the Brazilian Agricultural Research Corporation, 02°45'28 "N 60°43'54" W, located in the state of Roraima, in June, in the year 2015, with bioprocessed remaining (BR), seeds of fruit of *M. dubia*, from technological prospection, related to the years 2012, 2013 and 2014 in the northern Amazon.

Samples of the plant material – BR

The BR samples were obtained from 10 materials that were stored and preserved in a freezer since the collection period, 2012-2014.

Samples preparation

The BR samples were prepared from the formation of a series of sub-samples (1 kg) prepared from materials that were stored in the freezer, intending to obtain at least 500 g of processed sample according to granulometric specifications usually used for organic fertilizers. They were pre-dried in air circulating stove, calibrated in the range of 60 ± 5°C, for 48 h. After this period, they were weighted and processed using a Willye type mill with a 1 mm mesh, having previously calculated the efficiency of crushing and time required for processing.

From the processed material (suitably uniformed by granulometry), there were selected two samples, each containing 100 g to compose the biological assay in the laboratory, according to methodology principles of Zucconi et al. (1981) and Wong et al. (2001), with some adjustments.

Bioassay preparation

Here, a witness control (WC) (only deionized water) and aqueous extracts was prepared, including liquid substrates made from BR with and without treatment, known as T1 and T2, respectively. To obtain T2, it was placed 100 g of BR in a calibrated stove at a temperature of 200 ± 10°C for 2 h, in order to control the microbial population.

The experiment was conducted from preliminary results obtained in qualitative microbiological test conducted in a greenhouse with BR samples. In 76.9% treatments designed (solid substrates with different dosages in sand, sandy soil and clay soil), shown in a simplified way, treatment and identification in Table 1 revealed the presence of microorganisms in visual inspection from the third until the end, the seventh day of implementation of the experiment in all treatments containing the BR-intensive growth.

From the established treatments (T1 and T2), the biological assay was performed in specialized laboratory using sterilized materials, deionized water, ionic contaminants free and BR aqueous extracts, five repetitions for each witness control (WC) and treatments, obtained by means of two dilutions (D1 and D2) respectively defined by the ratio m: v, 1:10 normally used in the assessment of organic compounds and 1: 100 was performed as Sousa et al. (2015).

Once prepared, the extracts were shaken up manually for 20 s each, with a glass rod, repeating the action for three more times, in order to dissolve the material satisfactorily. Therefore, each extract was filtered through Whatman filter paper #1. Thereafter, simplified physicochemical evaluation, pH, electrical conductivity (EC) and total dissolved salts (TDS) was realized to obtain preliminary knowledge of the elaborated extracts. The measurement of pH, TDS and EC was determined by pH meter and microprocessor conductivity meter, after calibration with standard solution, according to the producer's instructions.

A model scheme was developed (Table 2) for simplified facilitation and identification of the experiment and extracts, used in the tabulation and presentation of results, as follows: RBST originated from untreated bioprocessed remaining (identified as T1) and RBCT originated from treated bioprocessed remaining (identified as T2), added to their respective dilution: T1D1 = 1:10; T1D2 = 1:100; T2D1 = 1:10; T2D2 = 1:100.

Table 2. Schematic model for simplified identification of the experiment and extracts made from bioprocessed remaining (BR).

Identification	Treatment	Dilution	Name simplified
RBST	T1	D1	T1D1
		D2	T1D2
RBCT	T2	D1	T2D1
		D2	T2D2

Bioassay installation

In laboratory bench, for each established treatment (Table 2) was added a qualitative analysis filter paper (Whatman #2) into five glass petri plate 9 cm in diameter, wetting with 5 ml of extract, each. For the WC, the same procedure was used, adding, in this case, 5 ml of deionized water per plate. Then, in each Petri dish were placed 10 *L. lycopersicum* seeds uniformly distributed.

The tomato seeds were acquired from agricultural store, after lifting of the most cultivated varieties in Roraima, also taking into consideration the characteristics of the “cultivar IPA 6”. According to information contained in the package, the IPA 6 has determined growth, vigorous and productive plant, firm (globular) fruit, with excellent internal and external color, as well as being resistant to disease such as *Fusarium*, *Verticillium*, nematode and Cracking. The germination takes place from 5 to 14 days after sowing.

Assessment of the bioassay

To obtain the results in the laboratory, visual inspection of the presence or absence of microorganisms and monitoring of the germination process of the seeds of the tomato proposed treatments was conducted for 120 h (five days), in a closed plate, by incubating the samples at 25 ± 1°C in a dark environment. At the first 120 h were recorded through photographic images and spreadsheet on the number of germinated seeds (NSG) and also by measuring with a caliper, root length (RL) of germinated seeds in each Petri dish. The seeds were considered to be germinated when they had rootlets and/or developed a little stem.

Germination index (GI)

The value for the germination index (GI) was obtained by quantifying the RSG and RRG, as proposed by Zucconi et al. (1981). Thus, the calculation of the RSG1s made by the equation:

RSG (%) = (NSG,T/NSG,C)*100

Where NSG,T is the arithmetic mean of the number of germinated seeds in each extract (treatment) and NSG,C is the arithmetic mean of the number of germinated seeds in witness control (WC).

The relative percentage of root length, RRG was obtained by the equation:

RRG (%) = (LR,T/LR,B)*100

Where LR, T is the mean length of the roots on the aqueous extract and LR,B is the length mean of the roots in the witness control(WC).

As was proposed by Zucconi et al. (1981), the germination index (GI), was computed using the equation:

Table 3. Methodological model proposed to quantify the degree or phytotoxicity level of the product from the tests with *Lycopersicon lycopersicum.*

GI (%)	Product rating under analysis
80-100	Non-phytotoxic
60-80	Moderately phytotoxic
30-60	Phytotoxic
< 30	Very phytotoxic

Source: Adapted from Trautmann and Krasny (1997) and WERL (2000).

GI (%) = (RSG %)*(RRG %)/100

Statistical analysis

The results were submitted to analysis of variance and means compared by Tukey test at 5% probability ($p < 0.05$) with the help of statistical program SISVAR® (Ferreira, 2011).

Degree qualification or phytotoxicity level of bioprocessed remaining, *M. dubia* seeds

The proposed methodological model is used (Table 3), from the GI (%), obtained after the use of the aqueous extracts with different treatments applied to biological assay with tomato was selected one that varies slightly among others found in the literature, adding small adjustments.

RESULTS AND DISCUSSION

In this study, preliminary knowledge were obtained related to the physical and chemical quality, presence of microorganisms in BR extracts of *M. dubia* seeds of northern Amazonia, also germination rate and degree of phytotoxicity (Table 4) of these from bioassay with *L. lycopersicum* (tomato), containing a control sample and four elaborated extracts from T1 treatments (RBST) and T2 (RBCT) in the proportion to 1:10 (D1) and 1:100 (D2).

In Table 4, it can be seen that all extracts (T1D1, T1D2, T2D1 and T2D2) tested showed pH values ranging from 4.73 to 5.18, below the obtained (5.83) with the witness control (WC), clearly indicating the influence of BR. Irrespective of treatment and applied dilution, T1D1 and T2D1 showed no significant difference related to pH, while T1D2 and T2D2 suffered directly influences in the applied dilution, differing from each other, and between WC and the other treatments performed with BR.

Analyzing the pH values obtained in the treated and untreated samples (Table 4), it is possible that pre-drying may have influenced the results, causing decay in pH values when compared to WC (Zanatta et al., 2010).

Minor pH occurred in extracts where BR was more concentrated (T1D1 and T2D1) (Table 4).

The results show important information about the influence of treatment of the bioprocessed compounds on

Table 4. Mean results obtained in the simplified physicochemical characterization, visual inspection of absence (-) or presence (+) of microorganisms, germination index and degree of phytotoxicity in the treatment with different doses of RBST and RBCT concentrated (10%) and diluted (1%).

Analyzed parameters	Treatment/Concentration				Attestation
	T1D1	T1D2	T2D1	T2D2	WC
pH	4.75±0.010^{d}	5.15±0.010^{c}	4.73±0.008^{d}	5.18±0.017^{b}	5.83±0.018^{a}
EC (dS m^{-1})	1.73±5.513^{a}	0.25±5.642^{c}	1.35±5.748^{b}	0.18±5.518^{d}	0.003±0.007^{e}
TDS (mg L^{-1})	951.50±3.390^{a}	135.63±3.470^{c}	742.17±3.535^{b}	100.49±3.393^{d}	2.05±0.046^{e}
Visual inspection	**+**	**+**	**-**	**-**	**-**
GI (%)	3.57	0.08	90.24	96.57	97.82
Phytotoxicity degree	Very phytotoxic	Very phytotoxic	Non-phytotoxic	Non-phytotoxic	Non-phytotoxic

Means followed by the same letters on the lines do not differ among them by Tukey test, at 5% probability.

Table 5. Average number of germinated seeds (NSG) (%) and average roots length (RL) (mm) of *Lycopersicon lycopersicum* (tomato), to five days after sowing (DAS) in different treatments (WC, T1D1, T1D2, T2D1 and T2D2).

Treatments/Parameters	WC	T1D1	T1D2	T2D1	T2D2
NSG (%)	92^{a}	84^{a}	24^{b}	88^{a}	90^{a}
RL (mm)	36.59^{a}	2.32^{b}	0.22^{b}	33.37^{a}	38.02^{a}

Means followed by the same letters on the lines do not differ among them by Tukey test, at 5% probability.

their chemical and physicochemical characteristics, which can alter their effects on the development of other plants. According to Chaves et al. (2004), the pH directly influences the development of microorganisms and temperature of sterilization, among other factors, corroborating to the data obtained, concerning to the classification of the degree of phytotoxicity found for the four treatments performed (Table 4).

During the visual inspection for the absence (-) or presence (+) of microorganisms in different doses of RBST and RBCT, after 3 days of seeding, it was observed on plates containing aqueous extract without treatment/sterilization, in the proportion to 1:10 to 1:100, a low rate of germination and visible presence of microorganism colonies, demonstrating the phytotoxicity. Dampened plates with deionized water (WC) and treated extracts already presented in this period, even on the third day, acceptable germination rate, values above 75%.

Generally, the germination capacity is often affected by the presence of pathogens inside or on the surface of the seeds (Souza et al., 2013). In this case, in T1D1, the most concentrated extract (proportion to 1:10), it was observed that the microorganisms present on the fifth day, not affect the germination process.

On other hand, in T1D2, the germination capacity of the seeds was affected (Table 5). In this case, the extract had become more diluted, in ratio to 1:100 as well as the BR used for the preparation of both extracts had not undergone any treatment, in addition to washing and pre drying stove. In T2D1 and T2D2 extracts, where there was a previous treatment, sterilization, it was observed that the germination of the seeds was not affected (Table 5).

Evaluation of the health of seeds enables development, more precisely, treatments to promote the elimination of present pathogens provide the restoration of health quality (Souza et al., 2013). Among the treatments, the phytotoxicity sources induced different effects regarding to the size and appearance of sunn hemp seedlings (Nunes et al., 2009). The same effect was observed, regarding to the appearance of roots of *L. lycopersicum*.

In Table 5 are shown results for the average number of germinated seeds (NSG) and average root length (RL) which led to the germination index (GI) of *L. lycopersicum* and its classification of phytotoxicity degree of the tested extracts (Table 4), obtained on the 5th day after sowing (DAS) in different treatments.

Of the four extracts evaluated among those who were previously treated, T2D1 and T2D2, regardless of the applied concentration, showed no phytotoxicity to the tested plant, being classified according to the germination index obtained (Figure 1).

The said extracts, T2D1 and T2D2 (Figure 1), when applied to substrates did not inhibit germination of the seeds of *L. lycopersicum* (Table 5) nor the growth of their roots. This may have occurred due to pH values presented (from 4.73 to 5.18) (Table 4) respectively acids to all the treatments, as well as the ionic behavior of the extracts (Figure 2).

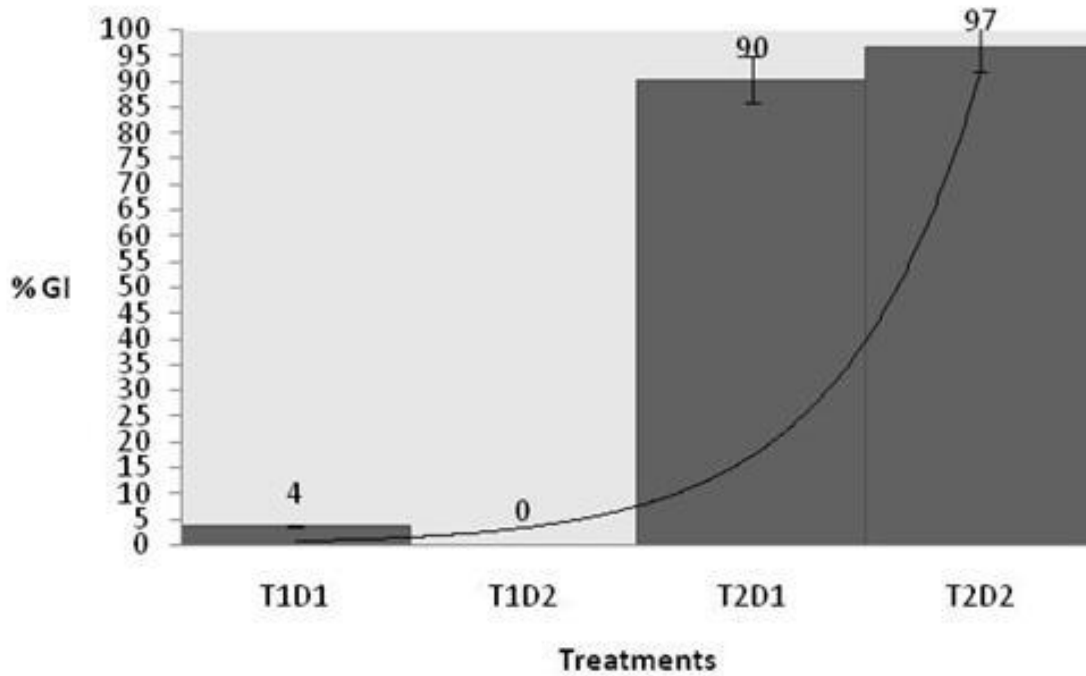

Figure 1. Germination Index (GI) of *L. lycopersicum* (tomato) obtained at 5 days after sowing (DAS) with *Myrciaria dubia* extracts of different treatments, T1D1, T1D2, T2D1 and T2D2 of BR.

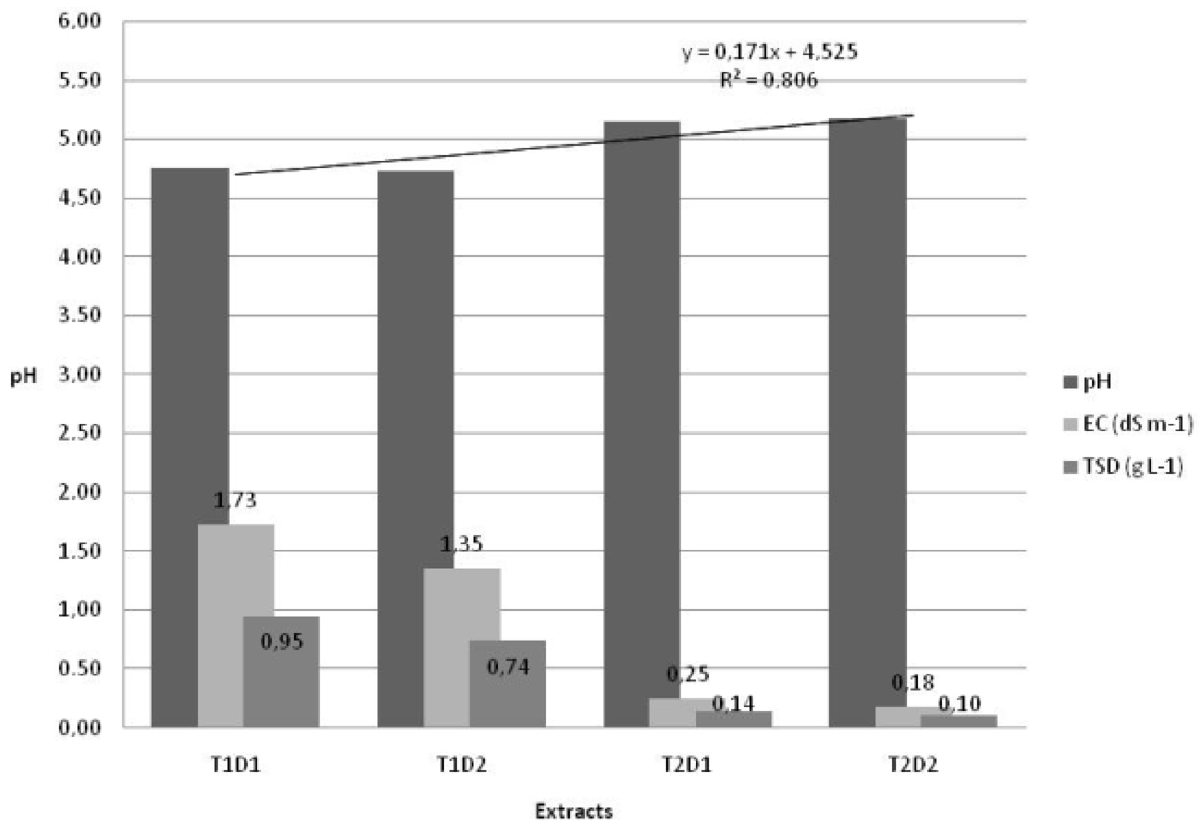

Figure 2. Demonstration related to pH trends, interrelated to electrical conductivity (EC) and total dissolved salts contents (TDS) of the four types extracts (T1D1, T1D2, T2D1 and T2D2) of bioprocessed remaining of *Myrciaria dubia* evaluated.

Figure 2 shows the ionic behavior of the extracts analyzed, T1D1 and T1D2 untreated and T2D1 and T2D2 with treatment when the pH values obtained are interrelated to electrical conductivity (EC) and total dissolved salts contents (TDSC).

The acidity and pH are considered important antimicrobial factors, providing greater stability to the product and to the microorganism's development (Souza et al., 2009). Despite of the presence of this natural barrier, the physiology of many yeasts and molds allows its adaptation to these adverse conditions, growing on substrates with intolerable sugar concentrations for bacteria, because they are not so sensitive to the high osmotic pressure. For these characteristics, Souza et al. (2006, 2009) found pH values ranging between 3.15 and 4.66. When evaluating the effect of particle size suspended in mango juice on the electrical conductivity, Vieira and Cartapatti-Stuchi (2006), concluded that the larger the particles on suspension, the lower will be the electrical conductivity, due to reduced ion mobility. This fact can be observed for EC results obtained in T1D1 and T2D1 (Figure 2).

From the mentioned results (Figure 2), it can be verified from data obtained by other authors, that the electric conductivity can be influenced by many parameters, such as temperature, electrolyte concentration, contents of chemical components, viscosity, solids in suspension, electrolytic strength and the presence of cellular structures (Min et al., 2007).

According to Lewicki (2004), it can be inferred that the electrical conductivity occurs in media containing electrically charged molecules Thus, as was mentioned by Min et al. (2007), *in situ* measurement of the electrical conductivity makes it possible to check the physical and chemical changes during the plant products transformation process.

The electrical conductivity (EC) reflects the salinity degree, which may indicate possible phytotoxic effects on the germination and growth of plants (Lin, 2008). Therefore, it can be a factor with a determining effect, mainly in the germination stage. For this study, it was found that the same trend, only with extracts which have not undergone any heat treatment. These showed relatively high electrical conductivity values (Figure 2), making possible its influence on the GI obtained; 3.6 and 0.1% for T1D1 T1D2 (Figure 1).

In this context, the results obtained from the analysis of electrical conductivity, total dissolved salts of aqueous extract at the beginning of the experiment and further visual inspection of the presence or absence of microorganisms, as well as the pH, facilitated the testing and evaluation of the phytotoxicity of bioprocessed remaining of *M. dubia* seeds.

Extracts derived from bioprocessed remaining, *M. dubia* seeds from the northern Amazon, including only those treated with thermally sterilized (T2D1 and T2D2) independent of the applied concentration, were not phytotoxic to the production of tomato.

Fracassetti et al. (2013) performed an evaluation of polyphenols, vitamin C content and antioxidant capacity of the dehydrated pulp, dry powder, the flour obtained from the peel and seeds, remaining residue after processing the *M. dubia* fruits. As result, fifty-three different phenolic compounds were determined, which were characterized by cutting-edge instruments. However, the content of phenolic compounds of remaining residue flour was higher than the pulp powder (4007.95 mg/100 g vs. 48.54 mg/100 g).

The flour is a rich source of bioactive compounds with potential health-promoting properties such as antioxidant, anti-inflammatory activity and hypocholesterolemic which have been linked to vitamin C and phenolic compounds such as flavonoids and ellagitannins. Nevertheless, according to the researchers, works *in vivo* and intervention studies are needed to assess the nutritional and functional potential of this product (Fracassetti et al., 2013).

According to Akter et al. (2011), Yuyama (2011) and Chagas et al. (2015), fruit of *M. dubia* (Caçari) is promising sources of various bioactive compounds like vitamin C, carotenoids and phenolic compounds. They are also good sources of potassium, iron, calcium, phosphorus and various types of amino acids, such as serine, valine and leucine. In this way, these evidences enable the following inferences, the presence of different bioactive compounds in this fruit can be used to retard or prevent various diseases such as cardiovascular disease and cancer (Akter et al., 2011).

The methodological process applied allowed a quick assessment, within 120 h, the aqueous extracts of *M. dubia* effect on seed germination and root growth of *L. lycopersicum.* From originated extracts of BR, those who were treated independent of the applied concentration, were not phytotoxic in the initial production of tomatoes.

Conclusions

The results show important information about the influence of treatment of the bioprocessed compounds on their chemical and physicochemical characteristics, which can alter their effects on the development of other plants. Therefore, there is possibility of availability of finished products and raw materials from the *M. dubia* seeds, since previously sterilized.

CONFLICT OF INTERESTS

The author has not declared any conflict of interests.

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ABBREVIATIONS

BR, Bioprocessed remaining; **S,** sand; **SS,** sandy soil; **CS,** clay soil; **EC,** electrical conductivity; **TDS,** total dissolved salts; **RBST,** untreated bioprocessed remaining; **RBCT,** treated bioprocessed remaining; **NSG,** number of germinated seeds; **RL,** root length; **GI,** germination index; **RSG,** relative percentage of germination; **DAS,** day after sowing.

REFERENCES

Akter MS, Oh S, Eun JB, Ahmed M (2011). Nutritional compositions and health promoting phytochemicals of camu-camu (*Myrciaria dubia*) fruit: A review. Food Res. Int. 44:1728-1732.

Araujo ASF, Monteiro RTR (2005). Plant bioassays to assess toxicity of textile sludge compost. Sci. Agric. 62(3):286-290.

Araujo GL, Campos MAA, Valente MAS, Silva SCT, França FD, Chaves MM, Tagliati CA (2014). Alternative methods in toxicity testing: the current approach. Braz. J. Pharm. Sci. 50(1):55-62.

Campos SC, Silva CG, Campana PRV, Almeida VL (2016). Toxicidade de espécies vegetais. Rev. Bras. Plantas Med. 18(1, Suppl. 1):373-382.

Chagas EA, Grigio ML, Durigan MFB, Fujita E, Vieites RL (2015). Caracterização centesimal e compostos bioativos de frutos de camu-camu em diferentes estádios de maturação. In. Congresso Brasileiro de Processamento mínimo e Pós-colheita de frutas, flores e hortaliças, 001. Anais. Aracaju-SE: (CD ROM), Maio de 2015.

Chaves MCV, Gouveia JPG, Almeida FAC, Leite JCAL, Silva FLH (2004). Caracterização físico-química do suco de acerola. Rev. Biol. Ciênc. Terra 4(2):123-157.

Cipollini D, Rigsby CM, Barto EK (2012). Microbes as target and mediators of allelopathy in plants. J. Chem. Ecol. 38(6):714-727.

Dias de Almeida G, Zucoloto M, Zetun MC, Coelho I, Sobreir FM (2008). Estresse oxidativo em células vegetais mediante aleloquímicos. Rev. Fac. Nac. Agron. Medellin 61(1):4237-4247.

Faria TM, Gomes Junior FG, Sa ME, Cassiolato AMR (2009). Efeitos alelopáticos de extratos vegetais na germinação, colonização micorrízica e crescimento inicial de milho, soja e feijão. Rev. Bras. Ciênc. Solo (online). 33(6)1625-1633.

Ferreira DF (2011). Sisvar: A computer statistical analysis system. Ciênc. Agrotec. 35(6):1039-1042.

Fracassetti D, Costa C, Moulay L, Barberán FAT (2013). Ellagic acid derivatives, ellagitannins, proanthocyanidins and other phenolics, vitamin C and antioxidant capacity of two powder products from camu-camu fruit (*Myrciaria dubia*). Food Chem. 139:578-588.

Habermann E, Imatomi M, Pereira VC, Pontes FC, Gualtieri SCJ (2015). Atividade fitotóxica de cascas do caule e folhas de *Blepharocalyx salicifolius* (MYRTACEAE) sobre espécies infestantes. Acta Biol. Colomb. 20(1):153-162.

Hall MH, Henderlong PR (1989). Alfalfa autotoxic fraction characterization and initial separation. Crop Sci. 29:425-428.

Imatomi M, Novaes P, Gualtieri SCJ (2013). Interespecific variation in allelopathic potential of the Myrtaceae family. Acta Bot. Bras. 27(1):54-61.

Lewicki PP (2004). Water as the determinant of food engineering properties. J. Food Eng. 61:483-495.

Lin C (2008). A negative-pressure aeration system for composting food wastes. Bioresour. Technol. 99:7651-7656.

Luz AC, Pretti IR, Dutra JCV, Batitucci MCP (2012). Avaliação do potencial citotóxico e genotóxico de Plantago major L. em sistemas teste *in vivo*. Rev. Bras. Plantas Med. (online) 14(4):635-642.

Mairesse LAS, Costa EC, Farias J, Fiorin RA (2007). Bioatividade de extratos vegetais sobre alface (*Lactuca sativa* L.). Rev. FZVA 12(2):1-12.

Min S, Sastry SK, Balasubramaniam VM (2007). In situ electrical conductivity measurement of select liquid foods under hydrostatic pressure to 800MPa. J. Food Eng. 82:489-497.

Moreno LF (1989). Efectos alelopaticos de *Rumex crispus* L. sobre *Pissum sativum* L. Acta Biol. Colomb. 1(5):35-43.

Noldin VF, Cechinel Filho V, Monache FD, Benassi JC, Christmann IL, Pedrosa RC, Yunes RA (2003). Chemical composition and biological activities of the leaves of *Cynara scolymus* L. (artichoke) cultivated in Brazil. Quím. Nova 26(3):331-334.

Nunes AS, Lourenção ALF, Pezarico CR, Scalon SPQ, Gonçalves MC (2009). Fontes e níveis de salinidade na germinação de sementes de *Crotalaria juncea* L. Ciênc. Agrotec. 33(3):753-757.

Ribeiro LO, Barbosa S, Balieiro FP, Beijo LA, Santos BR, Gouvea CMCP, Paiva LV (2012). Fitotoxicidade de extratos foliares de barbatimão [*Stryphnodendron adstringens* (Mart.) Coville] em bioensaio com alface. Rev. Bras. Bioci. 10(2):220-225.

Sausen TL, Löwe TR, Figueiredo LS, Buzatto CR (2009). Avaliação da atividade alelopática do extrato aquoso de folhas de *Eugenia involucrata* DC. e *Acca sellowiana* (O. Berg) Burret. Polibotanica 27:145-158.

Simoneto EL, Cruz-Silva CTA (2010). Alelopatia de sálvia sobre a germinação e o desenvolvimento do milho, tomate e girassol. Casc. 3(3):48-56.

Sousa RCP, Chagas EA, Guimarães PVP, Nascimento Filho WB, Melo Filho AA (2015). Minerals in Aqueous Extract of the Coproducts *Myrciaria dubia* (Kunth.) McVaugh, Myrtaceae. Rev. Virtual Quím. 7(4):1299-1305.

Souza BA, Marchini LC, Oda-Souza M, Carvalho CAL, Alves RMO (2009). Caracterização do mel produzido por espécies de *Melipona Illiger*, 1806 (apidae: meliponini) da região nordeste do Brasil: 1. Características físico-químicas. Quím. Nova 32(2):303-308.

Souza BA, Roubik DW, Barth OM, Heard TA, Enríquez E, Carvalho CAL, Villas-Bôas JK, Marchini LC, Locatelli JC, Persano-Oddo L, Almeida-Muradian LB, Bogdanov S, Vit P (2006). Composicion of stingless bee honey: Setting quality standards. Intercienc. Carc. 31:867-875.

Souza Filho APS, Guilhon GMSP, Santos LS (2010). Metodologias empregadas em estudos de avaliação da atividade alelopática em condições de laboratório: revisão crítica. Planta Daninha 28(3):698-697.

Souza LMS, Silva JB, Gomes NSB (2013). Qualidade sanitária e germinação de sementes de copaíba. Rev. Biosci. J. 29(1):1524-1531.

Trautmann NM, Krasny ME (1997). Composting in the Classroom, Scientific Inquiry for High School Students. Cornell University. Disponível em: <http://cwmi.css.cornell.edu/compostingintheclassroom.pdf>. Acesso em: nov. 2015.

Vieira JAG, Cartapatti-Stuchi GAS (2006). Efeito do tamanho das partículas e da tensão aplicada sobre a condutividade elétrica e o tempo de descongelamento do suco de manga. Sitientibus 35:99-109.

Weir TL, Park SW, Vivanco JM (2004). Biochemical and physiological mechanisms mediated by allelochemicals. Curr. Opin. Plant Biol. 7(4):472-479.

WERL - Woods End Research Laboratory (2000). Interpretation of waste and compost test. 1(4):1-6.

Wong J, Mak K, Chan N, Lam A, Fanf M, Zhou L, Wu Q, Liao X, (2001). Co-composting of soybean residues and leaves in Hong Kong. Bioresource Technol. 76(2):99-106.

Yuyama K (2011). A cultura de camu-camu no Brasil. Rev. Bras. Frut. 33(2)335-690.

Zanatta CL, Schlabitz C, Ethur EM (2010). Avaliação físico-química e microbiológica de farinhas obtidas a partir de vegetais não conformes à comercialização. Alim. Nutr. 21(3):459-468.

Zucconi F, Forte M, Bertoldi M (1981). Biological evaluation of compost maturity. Biocycle 22(4):27-29.

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Full Length Research Paper

Oversensitivity of Arabidopsis *gad1/2* mutant to NaCl treatment reveals the importance of GABA in salt stress responses

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Salt stress is one of the major problems in agricultural fields. Currently, more than 20% of irrigated agricultural lands are affected by salinity. High concentrations of sodium affects plant growth by competing with the uptake of important ions like potassium (K^+), and posing osmotic stress. Some plant species developed mechanisms such as modifying cellular metabolism to minimize effects of high salt concentrations. Gamma-aminobutyric acid (GABA) accumulation during salt stress is one of the results of modifications in cellular metabolism. However, whether this response is specific or not has not been shown before. Here, it was hypothesized that GABA accumulation is needed to counter the effects of salt stress. For that, GABA-depleted Arabidopsis *gad1/2* mutant was investigated for altered response under salt stress. Indeed, the double mutant was oversensitive to 150 mM NaCl treatment. Furthermore, the mutant was oversensitive to osmotic stress; since the double mutant showed reduced shoot water content after 300 mM mannitol treatment. Comparison of metabolites between salt-treated wild type and *gad1/2* mutant showed that GABA shunt plays a central role in modulating the carbon and nitrogen metabolism. Taken together, the findings show that GABA accumulation under salt stress conditions plays an important role to overcome the high salt concentration damage.

Key words: Salt stress, osmotic stress, GABA-shunt, tricarboxylic acid (TCA) cycle intermediates, potassium, transporters.

INTRODUCTION

Salinity is one of the several abiotic stresses that pose threat to the productivity of agricultural lands. Previous reports show that a quarter of irrigated agricultural lands worldwide are severely affected by salinity, and a further 1.5 million hectares of lands are abandoned every year because of salinity (Munns and Tester, 2008). There

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are several reasons for salinity problems in soils; however, irrigation water combined with poor drainage stand out as the most serious in damaging agricultural fields. High sodium level in soils affects plant growth in two ways; first, it increases the osmotic potential of the soils thereby reducing the uptake of water into roots (osmotic stress); second, it competes with the influx of important ions like K^+, inhibiting many physiological and biochemical processes such as nutrient uptake and assimilation (ionic stress) (Munns and Tester, 2008; Hasegawa et al., 2000). To counter the effects of salinity-induced cellular damages, plants have developed mechanisms such as osmotic tolerance and Na^+ exclusion (Munns and Tester, 2008). These tolerance mechanisms involve the interplay of several genes engaged in ion transport, detoxification process and metabolite production (Rus et al., 2001; Apse et al., 1999; Hayashi et al., 1997; Szekely et al., 2008).

Gamma-aminobutyric acid (GABA) is one of the metabolites that accumulate in response to abiotic stresses (Kinnersley and Turano, 2000). It is produced from the decarboxylation of glutamate by glutamate decarboxylase (GAD) within the cytosol and catabolized within the mitochondrial matrix by the activity of GABA-T and SSADH (Shelp et al., 2012). Despite the presence of five copies of *GAD* genes in Arabidopsis genome, two paralogs, *GAD1* and *GAD2*, are responsible for more than 90% of GABA produced in shoots and roots under normal growth conditions. Arabidopsis double mutants impaired in GAD1 and GAD2 functions contained low GABA in shoots and roots (Scholz et al., 2015; Mekonnen et al., 2016). However, the induction of *GAD4* transcripts under hypoxia (Miyashita and Good, 2008), drought (Urano et al., 2009) and cold (Kaplan et al., 2007) treatments suggest some specific functions of the GAD4 enzyme under stress conditions. Renault et al. (2010) also showed the induction of *GAD4* transcript under salt stress in a dose dependant manner and suggested the specificity of the response. However, whether this transcriptional induction of *GAD4* is important for GABA accumulation and salt stress tolerance of plants is still not clear.

GABA accumulation in response to salt stress has been reported in Arabidopsis (Renault et al., 2010), alfa alfa (Fougere et al., 1991), and tobacco (Binzel et al., 1987) plants. In Arabidopsis, GABA accumulation involves the up-regulation of almost all GABA-shunt genes at transcriptional level. Furthermore, the *in vitro* activity of GAD and GABA-T proteins were enhanced in response to salt stress (Renault et al. 2010). Despite the rapid accumulation of GABA under salt stress, little is known about its specificity and function under such conditions.

Renault et al. (2010) reported the specificity of the GABA shunt response under salt stress since, the *pop2-1* mutant impaired in GABA catabolism was over-sensitive to ionic stress. The authors concluded that the metabolism of GABA rather than GABA accumulation is needed to counter the ionic component of the salt stress. This provokes a question regarding the importance of GABA accumulation during salt stress. A recent report showed the importance of GABA accumulation than it's metabolism in drought stress response. GABA-depleted Arabidopsis *gad1/2* mutant exhibited oversensitive phenotype following drought stress treatment. Functional complementation with a third mutation in *pop2* gene specifically increased the GABA levels in shoots and reversed the drought over-sensitive phenotype of the double mutant (Mekonnen et al., 2016). The *pop2* mutation abolishes the GABA transaminase activity and leads to the accumulation of GABA.

The present work was conducted with the main objective of determining the importance of GABA accumulation under salt stress; first, by phenotypic characterization of GABA-depleted mutants under salt stress; second, by analyzing expression of major salt stress responsive genes; third, by analyzing the changes in C:N metabolism of mutant plants.

MATERIALS AND METHODS

Phenotypic analysis

Salt stress treatment

Seeds of wild type (Col-0) and *gad1/2* mutant (in Col-0 background) were sown on soil, cold treated and germinated in the greenhouse. Then, individual seedlings were transplanted to small pots filled with soil mix and kept in the greenhouse. The pots containing individual plants were arranged in a completely randomized design. Two weeks after transplanting, the salt stress treatment was initiated with 150 mM NaCl. The treatment was given by immersing the perforated tray containing pots in another tray filled with the salt solution.

Similarly, for control treatment plants on one of the trays were given water throughout the experiment. The treatment was given every third day for two weeks. Each treatment was replicated five times per genotype. The experiment was repeated once.

Osmotic stress treatment

Seeds from wild type and *gad1/2* mutant were sown and germinated in the green house as described previously. The seedlings were transplanted to pots filled with soil mix and transferred to growth chamber (16/8 light/dark cycle, ~70% RH). For osmotic stress treatment, four-week-old plants were watered without and with 300 mM mannitol. The experiment was arranged in a completely randomized design as described previously. For quantification of the shoot water content, shoot samples were collected beginning one day after the onset of the stress treatment. Determination of the shoot water content was carried out as described previously (Mekonnen et al., 2016). Each treatment was replicated five times per genotype.

RNA extraction, cDNA synthesis and qRT-PCR

Leaf materials were collected from control and 150 mM NaCl

treated four-week-old wild type and *gad1/2* plants, and snap-frozen in liquid nitrogen. RNA extraction was carried out as described previously (Scholz et al., 2015). RNase treatment, cDNA synthesis and qPCR were performed as described previously (Mekonnen et al., 2016). Primer pairs that specifically detect *GAD3* and *GAD4* transcripts were used (Renault et al., 2010). The primers used for specific amplification of *ABA2*, *ABA3*, *AAO3*, *AKT1*, *GORK*, *KAT1* and *HAK5* transcripts are listed in the appendix.

GC-MS and HPLC analysis of metabolites

GC-MS: Leaf materials (~100-200 mg) were collected from four-week-old wild-type and *gad1/2* plants treated without or with 150 mM NaCl, and immediately frozen in liquid nitrogen. Metabolite extraction and measurement was carried out as described previously (Scholz et al., 2015).

HPLC: Shoot and root samples were collected from four-week-old wild-type and *gad1/2* plants treated without or with 150 mM NaCl and immediately frozen in liquid nitrogen. For root sample collections, the entire plant was taken from the pot and soil was immediately removed by immersing the below soil part of the plant in water. Then, roots were briefly dried by placing them between tissue papers. The extraction and measurement of amino acids were performed as described previously (Mekonnen et al., 2016).

ICP MS measurement of Na^+ and K^+

At least 500 mg of fresh shoot materials were harvested from five-week-old wild-type and *gad1/2* Arabidopsis plants treated without and with 150 mM NaCl, and freeze dried in liquid nitrogen. The extraction and measurement of Na^+ and K^+ was carried out as described previously (Mekonnen et al., 2016).

Experimental design and statistical analysis

For phenotypic, chemotypic and expression analysis the experiments were arranged in a completely randomized design (CRD). For quantitative data, the statistical significance between treatment means was assessed using student's *t-test*.

RESULTS

Analysis of *GAD* transcripts and GABA accumulation

To determine the compensatory expression of *GAD3* and *GAD4* under salt stress, transcript abundances were investigated in shoots and roots of the *gad1/2* mutant. The salt stress treatment increased the abundance of *GAD4* transcripts in shoots of wild type plants, although it is not statistically significant (Figure 1A). In contrast, the abundance of *GAD4* transcript was unchanged in shoots of *gad1/2* mutant (Figure 1A). *GAD3* transcript was not detected in shoots and roots of both genotypes under normal growth conditions and was not induced either by salt stress treatment (Figure 1A and B). In roots of *gad1/2* mutant, the *GAD4* transcript was induced by nearly four-fold following the salt treatment. To determine if the induction of *GAD4* expression under salt stress was reflected at a metabolite level, the content of GABA in shoots and roots of both genotypes was determined. The GABA amount increased in shoots and roots of the wild type by 2- and 2.5-fold respectively, following the salt stress treatment (Figure 1C and D). However, in *gad1/2* shoots the GABA level increased to a detectable level following the salt stress treatment (Figure 1C). Surprisingly, in *gad1/2* roots the GABA content was not altered by the salt treatment, despite a four-fold increase in *GAD4* transcript (Figure 1D).

Phenotypic analysis of the *gad1/2* mutant under salt and osmotic stress conditions

The impact of GABA depletion on the phenotype of *gad1/2* mutant under salt stress was investigated. Interestingly, after two weeks of salt stress treatment *gad1/2* shoots were completely wilted, a characteristic of osmotic stress induced phenotype (Figure 2A). Ionic-stress induced features such as chlorosis and necrosis were not visible. To confirm the effects of the osmotic stress component, wild type and *gad1/2* plants were subjected to 300 mM mannitol. Under control conditions, the shoot water content was similar between the genotypes during four days of the osmotic stress treatment period (Figure 2B). However, mannitol treatment significantly reduced the shoot water content of the *gad1/2* mutant compared to the wild type (Figure 2B). This difference was evident on the third and fourth days after the initiation of the osmotic stress treatment.

Expression analysis of ABA biosynthetic genes

To examine if the salt stress in *gad1/2* mutant leads to induction of salt stress responsive genes, the transcript abundances of ABA synthesizing genes *ABA2*, *ABA3* and *AAO3* were analyzed. Both genotypes up-regulated the expressions of all three genes after 150 mM NaCl treatment (Figure 3A to C), confirming the occurrence of salt stress effects. The expression of *ABA3* was induced nearly by five-fold in shoots of *gad1/2* mutant following salt stress treatment (Figure 3B). The level of *ABA3* induction in *gad1/2* mutant was significantly greater than the wild type (~three-fold) after 150 mM NaCl treatment (Figure 3B). Similarly, the expression of *AAO3* was increased by four-fold in shoots of *gad1/2* mutant following the salt treatment and it is nearly double to the amount of transcripts measured in wild type shoots under the same treatment conditions (Figure 3C). At control conditions, there was no difference in the expression of all three genes between the genotypes (Figure 3).

Measurement of Na^+ and K^+ accumulations

To substantiate the salt oversensitive phenotype of *gad1/2*

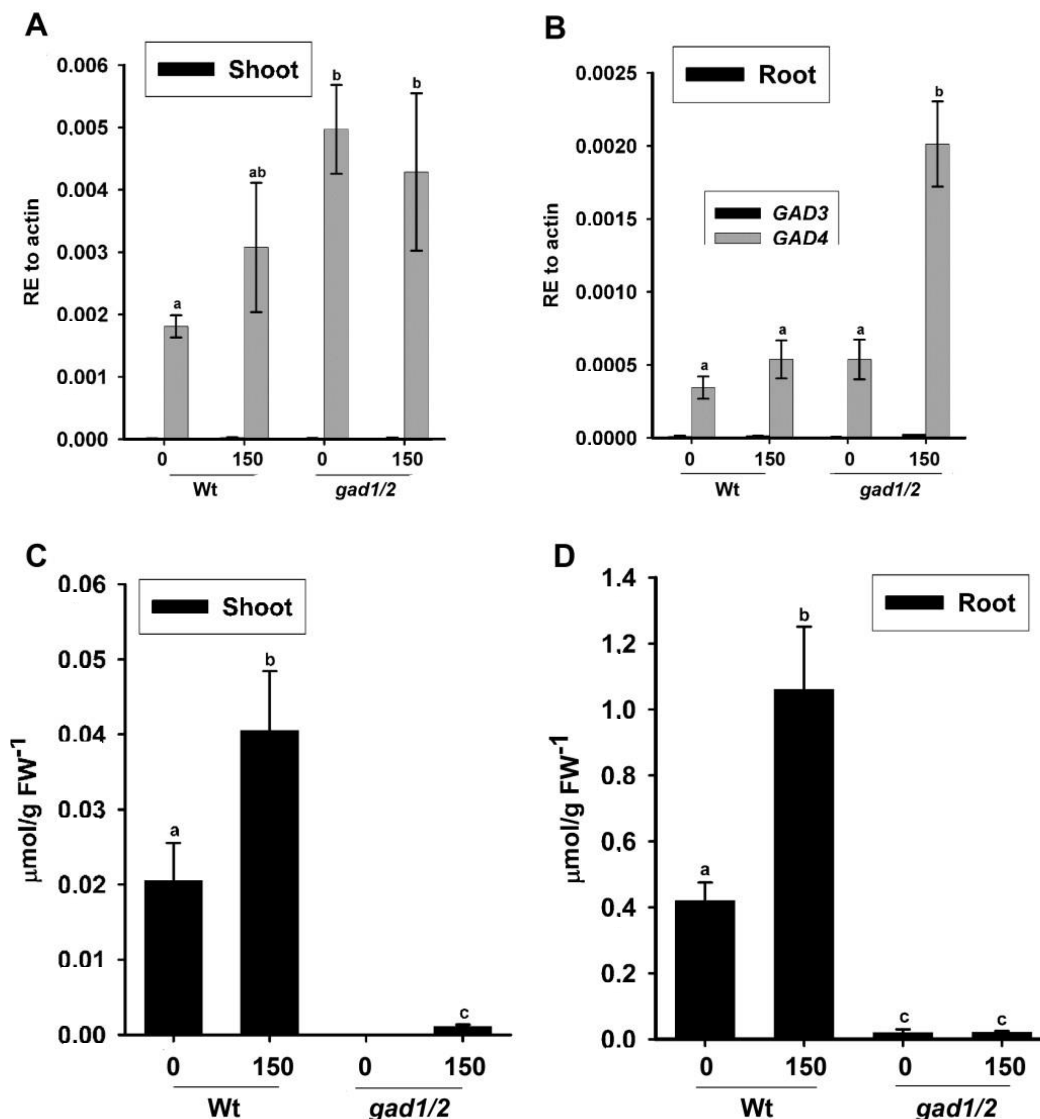

Figure 1. Analysis of *GAD* transcripts and GABA content in shoots and roots of four-week-old wild type and *gad1/2* mutants; transcript abundance of *GAD3* and *GAD4* in shoots (A) and root (B) of wild type and *gad1/2* mutants treated without and with 150 mM NaCl; GABA content in shoots (C) and roots (D) of wild type and *gad1/2* mutants treated without and with 150 mM NaCl; Values are means of three biological replicates for transcript analysis and at least five biological replicates for GABA measurement; error bars show the standard error of means; different letters on top of the error bars show significant differences between treatments after student's *t*-test, P<0.05.

mutant with ionic data, the Na^+ and K^+ amounts were determined in shoots. Both genotypes accumulated high amounts of sodium in shoots after 150 mM NaCl treatment; although, it was greater in *gad1/2* mutant (Figure 4A). In wild type plants the amount of sodium increased from 2.5 to 12 mg/g DW^{-1} whereas, in *gad1/2* mutant the sodium level increased from 2.5 to 20 mg/g DW^{-1} following salt stress treatment (Figure 4A). As expected, the potassium content was reduced in both genotypes after salt stress treatment, but it was sever in *gad1/2* mutant (Figure 4B). In *gad1/2* shoots, the level of potassium was reduced from 28 to 21 mg/g DW^{-1} which is statistically significant (Figure 4B). The combined effects of high sodium and less potassium accumulations in *gad1/2* mutant following salt treatment greatly affected the sodium and potassium homeostasis (Figure 4C).

Expression analysis of potassium transporters

To examine if low K^+ content in *gad1/2* mutant corresponds to the expression pattern of the potassium

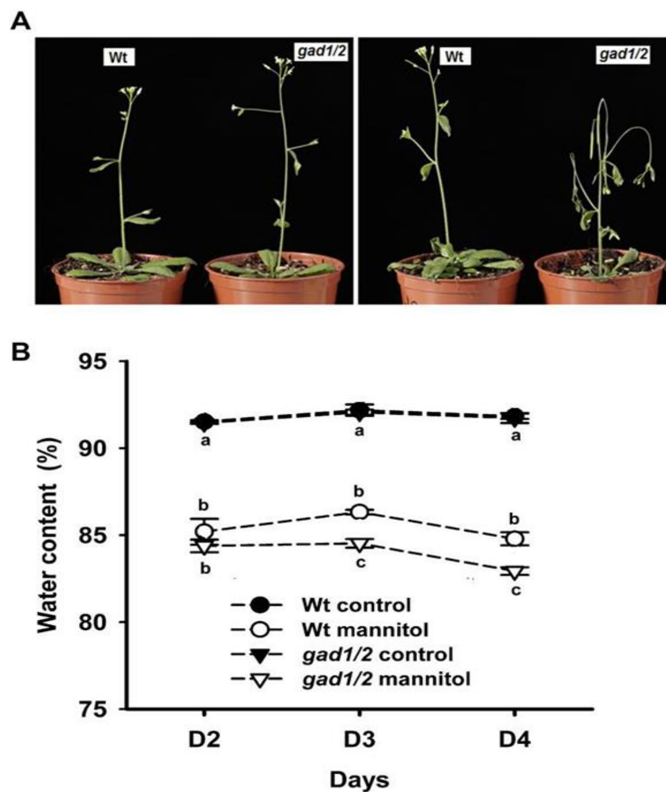

Figure 2. Phenotypic analysis of wild type and *gad1/2* mutants treated without and with 150 mM NaCl (A) and 300 mM mannitol (B); shoot phenotype of five-week-old wild type and *gad1/2* mutants treated without and with 150 mM NaCl for two weeks; pictures are representative of plants of similar phenotype; shoot water content measurement in wild type and *gad1/2* mutants treated without and with 300 mM mannitol; values are means of five biological replicates; different letters on top of the error bars show significant differences between treatments after student's *t*-test, P<0.05; D2 represent the second day after the initiation of the treatment.

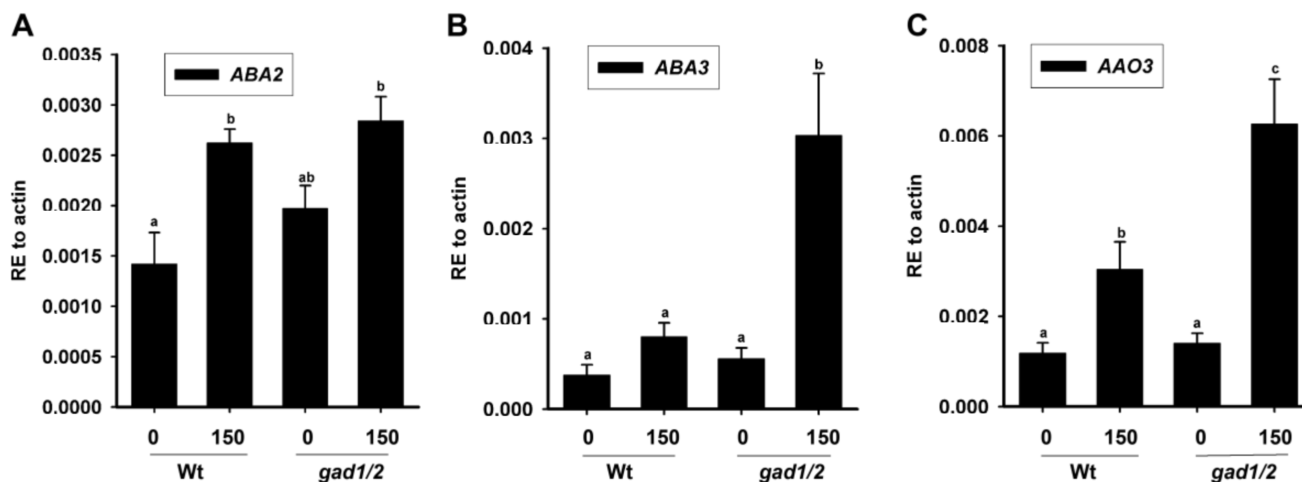

Figure 3. Transcript analysis of ABA synthesizing genes; the transcript abundances of *ABA2* (A), *ABA3* (B) and *AAO3* (C) genes were determined in shoots of four-weeks-old wild type and *gad1/2* mutants treated without and with 150 mM NaCl; values are means of three biological replicates; error bars show the standard error of means; different letters on top of the error bars show significant differences between treatments after student's *t*-test, P<0.05.

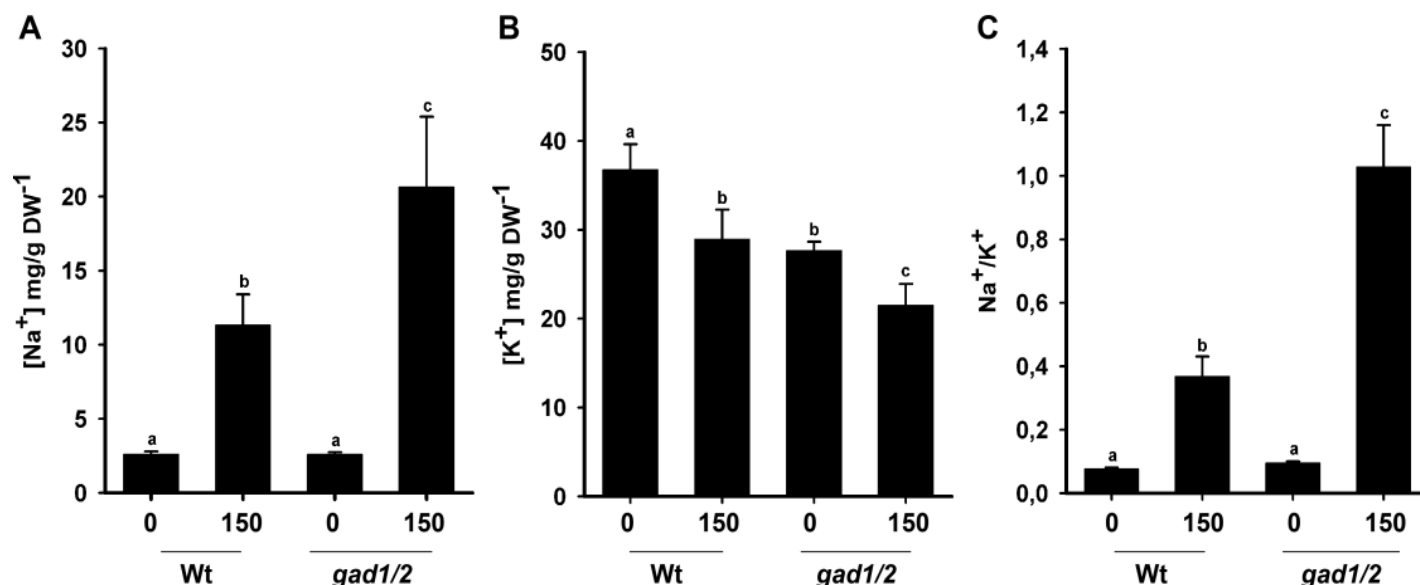

Figure 4. ICP-MS measurement of shoot Na^+ (A) and K^+ (B) contents in five-week-old wild type and *gad1/2* mutant treated without and with 150 mM NaCl; calculation of the Na^+/K^+ ratio (C); values are means of at least ten biological replicates; error bars show the standard error of means; different letters on top of the error bars show significant differences between treatments after student's *t*-test, P<0.05.

transporters, the transcript abundances of some potassium channels (KAT1, AKT1, GORK and HAK5) were analyzed. Under control conditions, there was no difference in shoot transcript abundances of *HAK5*, *KAT1* and *GORK* genes between the wild type and *gad1/2* mutant (Figure 5B to D). However, the expression of *AKT1* was reduced by four-fold in *gad1/2* shoots under control conditions (Figure 5A). In roots, the transcript abundances of all K^+ channels, except *GORK*, were similar between the genotypes under control conditions (Figure 5E, G&H). However, *GORK* transcript was three-fold higher in *gad1/2* roots under control conditions (Figure 5F). The treatment of 150 mM NaCl did not affect the expressions of all genes in shoots of the wild type (Figure 5A to D). However, in *gad1/2* mutant the transcript abundances of *HAK5* and *GORK* were altered by the salt treatment (Figure 5B and C). In roots of wild type plants, the expressions of *AKT1* and *GORK* were induced by the salt treatment (Figure 5E and F); whereas, in *gad1/2* mutant only *HAK5* transcript was significantly induced (Figure 5G).

Effects of salt treatment on the metabolic composition of *gad1/2* mutant

The importance of the GABA shunt in maintaining the balance of C and N metabolism under salt stress conditions was investigated. The accumulations of less abundant amino acids such as lysine, leucine and isoleucine were significantly increased in *gad1/2* shoots compared to the wild type after 150 mM NaCl treatment (Table 1). In contrast, the contents of most abundant amino acids like aspartate, glutamate and asparagine were not altered by the salt treatment (Table 1). The shoot glutamate content, 3.46 µg/g FW^{-1} in wt and 4.99 µg/g FW^{-1} in *gad1/2*, was significantly different between the two genotypes under control conditions; however, this difference was alleviated by the salt stress treatment (Table 1). Despite a considerable difference in shoot amino acid compositions between the wild type and *gad1/2* mutant under control conditions, the differences in roots were relatively minimum (Table 2). After salt stress treatment, the root amino acid contents of both genotypes were the same (Table 2). Investigation of the salt stress effect on TCA cycle intermediates revealed major changes. The contents of fumarate, oxaloacetate, malate and citrate were reduced by more than 50% (Figure 6A). In contrast, in *gad1/2* mutant the abundance of oxaloacetate, malate and citrate was increased by three or more-fold (Figure 6B). Surprisingly, after salt stress treatment 100-fold higher amount of proline was measured in shoots of *gad1/2* mutants compared to wild type. However, no proline was detected in shoots of both genotypes under normal growth conditions (Figure 6C).

DISCUSSION

Induction of *GAD4* transcript did not compensate for the loss of *gad1* and *gad2*

Arabidopsis genome contains five genes encoding glutamate decarboxylase (Shelp et al., 1999). These

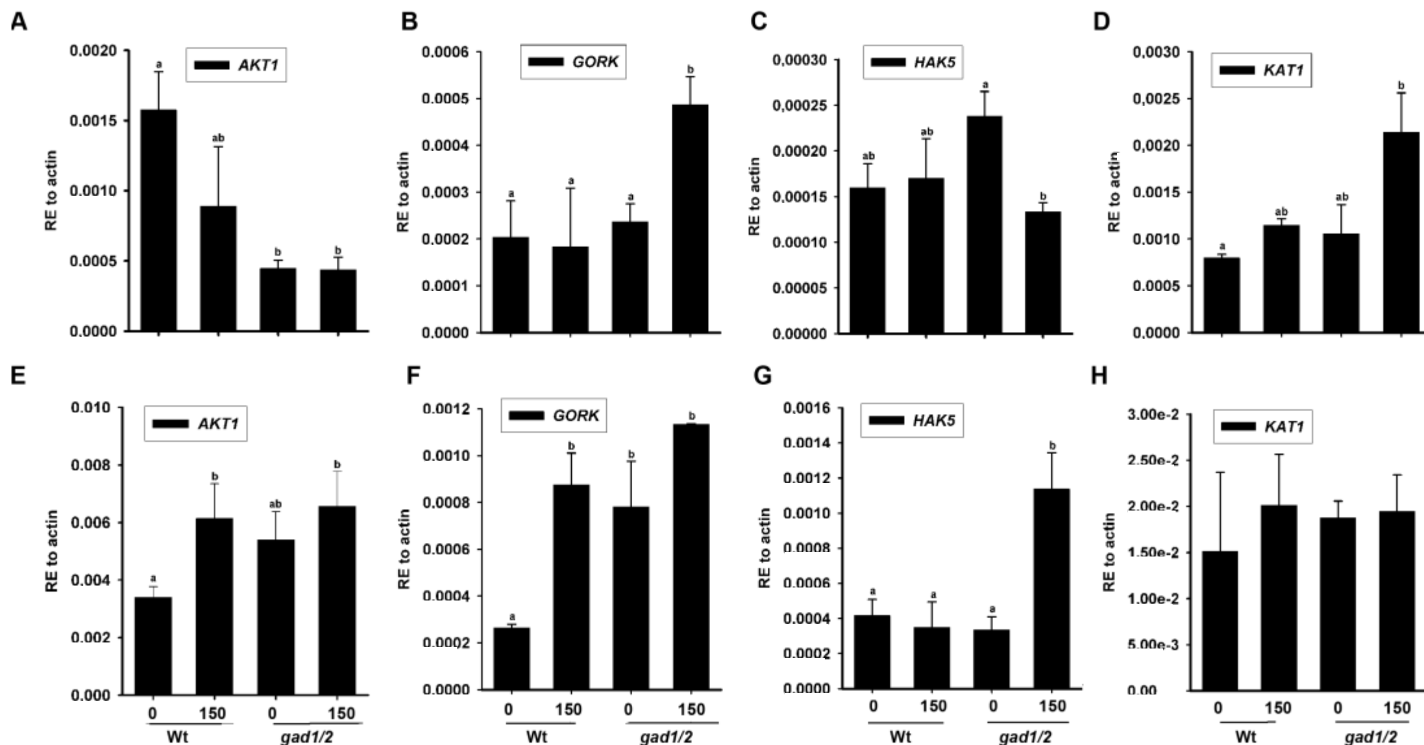

Figure 5. Transcript analysis of major potassium channels; the relative expression of *AKT1* (*A*), *GORK* (*B*), *HAK5* (C), *KAT1* (*D*) in shoots and *AKT1* (*E*), *GORK* (*F*), *HAK5* (*G*), *KAT1* (*H*) in roots of four-week-old wild type and *gad1/2* mutants treated without and with 150 mM NaCl; the expressions were normalized against actin; values are means of three biological replicates; error bars show the standard error of means; different letters on top of the error bars show significant differences between treatments after student's *t*-test, P<0.05, RE-relative expression.

Table 1. HPLC measurement of amino acids (µmol/g FW) in shoots of four-week-old wild type and *gad1/2* mutant treated without and with 150 mM NaCl.

Amino acids	Shoot			
	Control		150 mM NaCl	
	Wt	*gad1/2*	Wt	*gad1/2*
Asp	2.105±0.033	3.879±0.256*	2.631±0.143	3.462±0.416 ^{ns}
Glu	3.458±0.083	4.992±0.321*	4.621±0.240	6.257±0.837^{ns}
Ser	0.216±0.018	0.253±0.025^{ns}	0.246±0.014	0.340±0.050^{ns}
Asn	1.411±0.113	0.985±0.108*	3.012±0.096	3.798±0.529^{ns}
Gln	1.073±0.119	1.291±0.105 ^{ns}	1.230±0.082	1.227±0.178 ^{ns}
Gly	0.045±0.010	0.065±0..021^{ns}	0.035±0.002	0.040±0.006^{ns}
Thr	0.468±0.042	0.643±0.057*	0.427±0.031	0.636±0.105^{ns}
Ala	0.497±0.041	0.512±0.057^{ns}	0.478±0.035	0.415±0.064^{ns}
Arg	0.031±0.001	0.035±0.004^{ns}	0.036±0.001	0.054±0.008*
Tyr	0.017±0.001	0.015±0.002^{ns}	0.030±0.002	0.065±0.010*
Val	0.091±0.004	0.103±0.008^{ns}	0.107±0.004	0.177±0.025*
Trp	0.009±0.001	0.010±0.001^{ns}	0.014±0.001	0.028±0.004*
Phe	0.027±0.001	0.032±0.003^{ns}	0.039±0.002	0.063±0.010*
Ileu	0.048±0.004	0.040±0.003^{ns}	0.056±0.002	0.091±0.013*
Leu	0.044±0.002	0.041±0.004^{ns}	0.065±0.004	0.114±0.017*
Lys	0.035±0.002	0.046±0.003*	0.046±0.003	0.099±0.014*

*Values are means of at least six biological replicates; asterisks show statistical significance after student's t-test; * P<0.05, ns-non significant.

Table 2. HPLC measurement of amino acids (μmol/g FW) in roots of four-week-old wild type and *gad1/2* mutant treated without and with 150 mM NaCl.

Amino acids	Root			
	Control		150 mM NaCl	
	Wt	*gad1/2*	Wt	*gad1/2*
Asp	0.233±0.033	0.250±0.015^{ns}	0.538±0.081	0.478±0.053^{ns}
Glu	0.618±0.105	1.442±0.136*	1.725±0.238	2.311±0.229^{ns}
Ser	0.122±0.023	0.164±0.024^{ns}	0.355±0.052	0.394±0.055^{ns}
Asn	0.213±0.057	0.235±0.038^{ns}	0.908±0.089	1.016±0.093^{ns}
Gln	0.391±0.084	0.462±0.057^{ns}	1.271±0.176	1.191±0.126^{ns}
Gly	0.047±0.007	0.035±0.004^{ns}	0.085±0.012	0.077±0.006^{ns}
Thr	0.138±0.022	0.171±0.012^{ns}	0.411±0.035	0.367±0.029^{ns}
Ala	0.237±0.026	0.336±0.071^{ns}	0.540±0.099	0.445±0.046^{ns}
Arg	0.004±0.001	0.034±0.023^{ns}	0.009±0.002	0.012±0.001^{ns}
Tyr	0.011±0.002	0.012±0.001^{ns}	0.025±0.003	0.023±0.002^{ns}
Val	0.040±0.006	0.060±0.005*	0.124±0.015	0.104±0.007^{ns}
Trp	0.058±0.007	0.008±0.001*	0.102±0.019	0.041±0.004*
Phe	0.013±0.004	0.010±0.001^{ns}	0.035±0.004	0.028±0.002^{ns}
Ileu	0.020±0.003	0.027±0.003^{ns}	0.046±0.005	0.038±0.004^{ns}
Leu	0.024±0.003	0.040±0.004*	0.066±0.006	0.062±0.008^{ns}
Lys	0.011±0.001	0.010±0.001^{ns}	0.022±0.004	0.023±0.006^{ns}

* Values are means of at least six biological replicates; asterisks show statistical significance after student's *t*-test; * P<0.05; ns, non significant

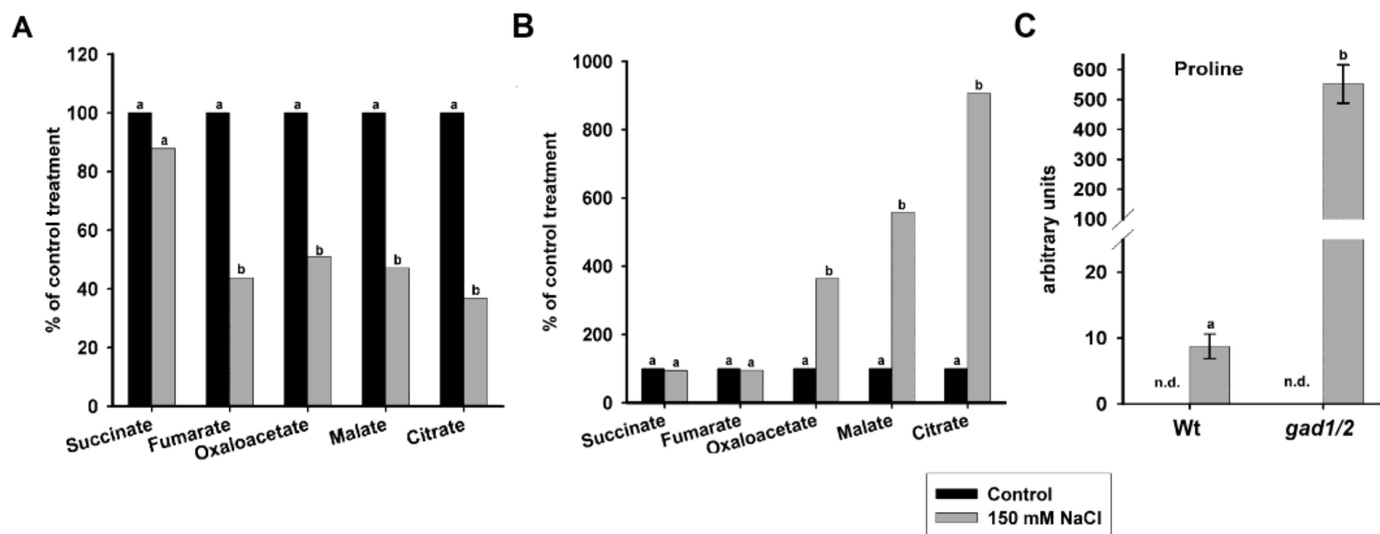

Figure 6. GC-MS measurement of TCA cycle intermediates and proline; the shoot TCA cycle intermediates in four-week-old wild type (A) and *gad1/2* (B) plants treated without and with 150 mM NaCl; proline content in shoots of wild type and *gad1/2* mutants treated without and with 150 mM NaCl (C); values are means of at least eight biological replicates; error bars in (C) show the standard error of means; different letters on top of the error bars show significant differences between treatments after student's *t*-test, P<0.05; n.d., not detected.

five paralogs show organ specificity in terms of expression (Shelp et al., 2012). For example, *GAD1* expresses mainly in roots, whereas *GAD2* transcript was detected in all organs (Zik et al., 1998). Similar to *GAD2*, *GAD4* transcript was detected in various organs such as shoots, roots, flowers and siliques (Scholz et al., 2015; Renault et al., 2010). The induction of *GAD4* expression in response to salt stress (Renault et al.,

2010), hypoxia (Miyashita and Good, 2008), drought stress (Urano et al., 2009) and cold treatment (Kaplan et al., 2007) has been shown previously. In the present work, *GAD4* transcript was induced in wild type plants treated with 150 mM NaCl, an observation in line with a previous report (Renault et al., 2010). The significant induction of *GAD4* transcript in roots of *gad1/2* mutant after salt stress treatment might suggest the severity of the stress in the *gad1/2* mutant. Despite the induction of *GAD4* expression in shoots and roots of the *gad1/2* mutant, its effect was not reflected at the metabolite level. This probably suggests a minor contribution of GAD4 to the total GABA pool in tissues. To date, only few metabolic pathways are known to be regulated at transcriptional level (Urano et al., 2009).

GABA synthesis in plants is regulated post-translationally by the Ca^{2+}/CaM complex (Bouché et al., 2004). Therefore, the induction of *GAD4* expression may not necessarily lead to high GABA levels.

The *gad1/2* mutant is oversensitive to the salt and osmotic stress

The induction of GABA shunt activities and GABA accumulation in response to various abiotic stresses such as drought, salt, cold and hypoxia have been reported previously (Kinnersley and Turano, 2000; Miyashita and Good, 2008). Subsequently, the phenotypes of some GABA-shunt mutants were characterized under abiotic stress conditions. Renault et al. (2010) examined the responses of GABA-rich *pop2-1* mutants to salt stress treatments and observed oversensitivity to only the ionic component of the salt stress. Recently, GABA-depleted *gad1/2* mutant was characterized under drought stress conditions and exhibited over-sensitive phenotype compared to the wild type (Mekonnen et al., 2016). Here, the responses of the *gad1/2* mutants to 150 mM NaCl treatment were investigated. Interestingly, the mutant plants exhibited a wilty-phenotype as shown by the loss of turgor and bending of the inflorescence. Salt stress generates different types of effects due to its osmotic and ionic components. The osmotic stress effect is usually rapid and manifested earlier than the ionic component (Munns et al., 1995). In the present work, the osmotic stress component of the salt stress treatment was visible two weeks after the onset of the treatment, as shown by the bending of inflorescences and shrinking of leaves. Furthermore, the lower shoot water content in *gad1/2* mutant following 300 mM mannitol treatment suggests the greater effect of the osmotic stress. GABA is one of the most abundant amino acids in roots and accounts up to 7% of the total free amino acids under normal growth conditions (Bouché et al., 2004). The increment of GABA amount by two-fold in wild type roots following salt stress (Figure 1D) probably suggests the importance of GABA in defense against the osmotic stress effect. The function of GABA as osmoticum has been suggested previously (Bown and Shelp, 1997). Furthermore, high GABA accumulating Arabidopsis *pop2* mutants showed tolerance to mannitol induced osmotic stress (Renault et al., 2010). Similarly, exogenous application of GABA to maize seedlings increased the endogenous GABA level and subsequently improved tolerance to salt stress (Wang et al., 2017). Sensitivity to the ionic component of salt stress requires the accumulation of Na^+ to a toxic level. For example, salt overly sensitive (*sos3*) mutants treated with 100 mM NaCl accumulated 60 mg/g DW^{-1} Na^+ (Zhu et al., 2007). Similarly, the salt oversensitive *pop2-1* seedlings accumulated ~50 mg/g DW^{-1} of sodium after 150 mM NaCl treatment (Renault et al., 2010). In *gad1/2* mutant, the amount of sodium ion might have increased by 8-fold after 150 mM NaCl treatment however; the concentration (20 mg/g DW^{-1}) was comparatively smaller than the amount measured in salt sensitive mutants. The measurement of lower quantity of Na^+ in *gad1/2* mutant after salt stress treatment may not necessarily reflect an alteration in sodium uptake from the soil. It could also be due to a difference in treatment conditions and age of the plant. In fact, GABA has been shown to involve in the uptake of Na^+. Arabidopsis seedlings treated with 1 and 10 mM exogenous GABA accumulated more Na^+ than the control (Essah et al., 2003). However, in the present work low endogenous GABA of *gad1/2* mutant did not correspond to low sodium level. Similarly, high endogenous GABA accumulating *pop2-1* mutant accumulated similar amount of sodium compared to the wild type following NaCl treatment (Renault et al., 2010). These observations suggest that endogenous GABA do not influence sodium uptake in plants. In contrast, potassium levels seem to have correlation with the endogenous GABA level. High GABA containing *pop2* mutants accumulated more K^+ ion (Renault et al. 2010) than low GABA containing *gad1/2* mutant (Figure 4B). Despite a notable change in Na^+/K^+ ratio of *gad1/2* mutant following salt stress, the ionic stress symptoms were not visible within the experimental period probably due to the rapid and severe effect of the osmotic stress.

The severity of the salt stress effect in *gad1/2* mutant was reflected by the significant increase in transcription of ABA synthesizing genes. Plants accumulate ABA in vegetative tissues when encounter adverse environmental conditions such as drought and salt (Xiong and Zhu, 2003; Zhu, 2002; Seo and Koshiba, 2002). Kefu et al. (1991) showed the rise of ABA levels in barley and cotton leaves following treatment of 75 mM NaCl. Here, the transcripts of ABA synthesizing genes (ABA3 and AAO3) were significantly induced in *gad1/2* mutant following 150 mM NaCl treatment, indicating the severity of the stress. ABA3 and AAO3 are involved in the last step of the ABA biosynthesis pathway (Seo et al., 2000; Bittner et al., 2001). Despite the lack of sufficient

experimental evidences on the interaction between the GABA shunt and ABA pathway, the proper functioning of the ABA pathway was required for the GABA effect on 14-3-3 expressions (Lancien and Roberts, 2006).

GABA-depletion differentially regulated the expressions of *AKT1* and *GORK* in *gad1/2* mutants

Potassium is one of the most important and abundant cation in plants, and accounts up to 10% of the plant dry matter (Leigh and Wyn Jones, 1984). It plays a central role in many biological processes therefore; it is required in large quantity. To meet this requirement, plants should posses an effective mechanism that involves potassium channels to absorb potassium from the medium through their roots and translocate to the aerial parts (Gierth and Mäser, 2007). In Arabidopsis, three major potassium transporter family proteins, in addition to channels, have been identified (Maser et al., 2001). Despite the existence of diverse channels and transporters in Arabidopsis, two proteins, AtAKT1 and AtHAK5, contribute 84 and 78% of low affinity and high affinity transport of potassium, respectively, in wild type roots (Gierth and Mäser, 2007). In the present work, the difference in shoot K+ content between the Wt and *gad1/2* mutant was surprising considering the similarity in root *AKT1* and *HAK5* expressions under control conditions. In fact, the transcript data alone may not fully explain the difference in shoot K^+ level. The difference could also be due to a change in its translocation to shoots. AKT1 has been shown to involve in potassium retrieval from xylem sap (Lagarde et al., 1996). The down regulation of *AKT1* expression in GABA-depleted *gad1/2* shoots probably reduced the potassium retrieval from xylem which in turn has a feedback effect on the loading of potassium to the xylem vessel in roots. Furthermore, the up-regulation of *GORK* expression in shoots and roots of *gad1/2* mutant without and with 150 mM NaCl treatment, respectively, probably led to the efflux of potassium from the plant. Previous works showed that GORK effluxes potassium from roots to the medium, and the process is enhanced under stress conditions (Demidchik, 2014; Jayakannan et al., 2013). The enhancement of GORK activity under salt stress is associated with the depolarization of the plasma membrane due to the uptake of more Na^+. Alternatively, the reduced accumulation of potassium in *gad1/2* mutant could be due to the lack of GABA-inhibition effect on anion channels like ALMT. The efflux of anions depolarizes membranes and this leads to inhibition of K^+ uptake. The negative regulation of ALMT proteins by GABA has been reported recently (Ramesh et al. 2015). In general, the low potassium content in shoots of *gad1/2* mutant could be the combined effects of reduced potassium retrieval from the xylem, an efflux of potassium from roots and reduced uptake from the soil.

Disruption of the GABA-shunt altered the stress-induced modification of cellular metabolism in *gad1/2* mutant

Plants respond to environmental stresses with various kinds of cellular responses such as modification of cell wall architecture, adjustment to membrane system and changes in cell cycle and cell division (Krasensky and Jonak, 2011). Modification in cellular metabolism is also a response that plants undertake during abiotic stresses. Alfalfa roots exposed to 100 and 150 mM NaCl accumulated more amino acids and less organic acids compared to the control (Fougére et al., 1991). Similarly, Arabidopsis plants accumulated more amino acids and less carbohydrate when treated with 150 mM NaCl (Renault et al., 2010). In the present work, the accumulations of amino acids were generally increased in shoots and roots of both genotypes after NaCl treatment (Tables 1 and 2). However, the increment was significant in shoots of *gad1/2* mutants than the wild type. The rise of amino acid levels under stress conditions could come from production or stress-induced protein degradation (Krasensky and Jonak, 2011).

Glutamate is a precursor for many of the amino acids produced in cells (Bouché and Fromm, 2004). In wild type plants, the glutamate content was increased by 44 and 133% in shoots and roots, respectively, after 150 mM NaCl treatment. Therefore, it is not surprising to see an increment in amino acid levels. In *gad1/2* mutant, the glutamate content was increased by 35 and 34% in shoots and roots, respectively, after salt treatment. With a relatively smaller change of glutamate content, the accumulation of amino acids in *gad1/2* shoots might come from the degradation of proteins induced by stress. Besides, the disruption of the GABA-shut also affects the TCA cycle intermediate. The direct link between the GABA-shunt and the TCA cycle has been shown in tomato (Studart-Guimaraes et al., 2007) and potato (Araújo et al., 2008). The reduction of organic acids (Fougére et al., 1991) and accumulation of GABA (Renault et al., 2010) under salt stress suggests that GABA-shunt is the preferred route for C and N metabolism under such conditions. The increment of organic acid levels in *gad1/2* mutants following salt treatment further confirms the importance of the GABA-shunt under stress conditions. Proline is another compound that accumulates in response to stresses (Singh et al., 1972; Renault et al., 2010). Despite a modest increase in wild type plants following salt stress, the amount of proline was remarkably increased in *gad1/2* mutant. In wild type plants, GABA-shunt seems to be the preferred route for glutamate metabolism than proline syntheses under salt stress condition. Such preferential accumulation of GABA over proline has been

reported in tobacco plants exposed to water stress (Liu et al., 2011). GABA and proline are synthesized from a common precursor, glutamate (Krasensky and Jonak, 2011). These observations suggest that GABA plays a central role in salt induced modifications of cellular metabolism.

Taken together, the accumulation of GABA under salt stress plays a certain role to counter salt induced damage. However, further experiments should be conducted to determine the specific function of GABA under salt stress conditions.

CONFLICT OF INTERESTS

The author has not declared any conflict of interest.

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REFERENCES

Apse MP, Aharon GS, Snedden WA, Blumwald E (1999). Salt tolerance conferred by overexpression of a vacuolar Na^+/H^+ Antiport in Arabidopsis. Science 285(5431):1256-1258.

Araújo WL, Nunes-Nesi A, Trenkamp S, Bunik VI, Fernie AR (2008). Inhibition of 2- oxoglutarate dehydrogenase in potato tuber suggests the enzyme is limiting for respiration and confirms its importance in nitrogen assimilation. Plant Physiol. 148:1782-1796.

Binzel ML, Hasegawa PM, Rhodes D, Handa S, Handa AK, Bressan RA (1987). Solute accumulation in tobacco cells adapted to NaCl. Plant Physiol. 84(4):1408-1415.

Bittner F, Oreb M, Mendel RR (2001). ABA3 is a molybdenum cofactor sulfurase required for activation of aldehyde oxidase and xanthine dehydrogenase in *Arabidopsis thaliana.* J Biol. Chem. 276:40381-40384.

Bouché N, Fromm H (2004). GABA in plants: just a metabolite? Trends Plant Sci. 9:110-115.

Bouché N, Fait A, Zik M, Fromm H (2004). The root-specific glutamate decarboxylase (GAD1) is essential for sustaining GABA levels in Arabidopsis. Plant Mol. Biol. 55:315-325.

Bown AW, Shelp BJ (1997). The metabolism and functions of γ-aminobutyric acid, Plant Physiol. 115:1-5.

Demidchik V (2014). Mechanisms and physiological roles of K^+ efflux from root cells. J. Plant Physiol. 171(9):696-707.

Essah PA, Davenport R, Tester M (2003). Sodium influx and accumulation in Arabidopsis. Plant Physiol. 133(1):307-318.

Fougére F, Le Rudulier D, Streeter JG (1991). Effects of salt stress on amino acid, organic acid, and carbohydrate composition of roots, bacteroids, and cytosol of alfalfa (*Medicago sativa* L.). Plant Physiol. 96:1228-1236.

Gierth M, Mäser P (2007). Potassium transporters in plants-involvement in K^+ acquisition, redistribution and homeostasis. FEBS Lett. 581:2348-2356.

Hasegawa PM, Bressan RA, Zhu JK, Bohnert HJ (2000). Plant cellular and molecular responses to high salinity. Annu. Rev. Plant Physiol. Plant Mol. Biol. 51:463-499.

Hayashi H, Mustardy L, Deshnium P, Ida M, Murata N (1997). Transformation of *Arabidopsis thaliana* with the *codA* gene for choline oxidase; accumulation of glycine betaine and enhanced tolerance to salt and cold stress. Plant J. 12:133-142.

Jayakannan M, Bose J, Babourina O, Rengel Z, Shabala S (2013). Salicylic acid improves salinity tolerance in *Arabidopsis* by restoring membrane potential and preventing salt-induced K^+ loss via a GORK channel. J. Exp. Bot. 64(8):2255-2268.

Kaplan F, Kopka J, Sung DY, Zhao W, Popp M, Porat R, Guy CL (2007). Transcript and metabolite profiling during cold acclimation of Arabidopsis reveals an intricate relationship of cold-regulated gene expression with modifications in metabolite content. Plant J. 50:967-981.

Kefu Z, Munns R, King RW (1991). Abscisic acid levels in NaCl treated barley, cotton, and saltbush. Aust. J. Plant Physiol. 18:17-24.

Kinnersley AM, Turano FJ (2000). γ-Aminobutyric acid (GABA) and plant responses to stress. Crit. Rev Plant Sci. 19:479-509.

Krasensky J, Jonak C (2012). Drought, Salt and Temperature stress-induced metabolic rearrangements and regulatory networks. J. Exp. Bot. 63(4):1593-608.

Lagarde D, Basset M, Lepetit M, Conejero G, Gaymard F, Astruc S, Grignon C (1996). Tissue-specific expression of Arabidopsis *AKT1* gene is consistent with a role in K^+ nutrition. Plant J. 9(2):195-203.

Lancien M, Roberts MR (2006). Regulation of *Arabidopsis thaliana* 14-3-3 gene expression by γ- aminobutyric acid. Plant Cell Environ. 29:1430-1436.

Leigh RA, Wyn Jones RG (1984). A hypothesis relating critical potassium concentrations for growth to the distribution and function of this ion in the plant cell. New Phytol. 97:1-13.

Liu C, Zhao L, Yu G (2011). The dominant glutamic acid metabolic flux to produce *γ*- aminobutyric acid over proline in *Nicotiana tabacum* leaves under water stress relates to its significant role in antioxidant activity. J. Integr. Plant Biol. 53:608-618.

Maser P, Thomine S, Schroeder JI, Ward JM, Hirschi K, Sze H, Talke IN, Amtmann A, Maathuis FJ, Sanders D, Harper JF (2001). Phylogenetic relationships within cation transporter families of arabidopsis. Plant Physiol. 126:1646-1667.

Mekonnen DW, Fluegge UI, Ludewig F (2016). Gamma-aminobutyric acid depletion affects stomata closure and drought tolerance of *Arabidopsis thaliana*. Plant Sci. 245:25-34.

Miyashita Y, Good AG (2008). Contribution of the GABA shunt to hypoxia induced alanine accumulation in roots of *Arabidopsis thaliana*. Plant Cell Physiol. 49:92-102.

Munns R, Schachtman DP, Condon AG (1995). The significance of a two-phase growth response to salinity in wheat and barley. Aust. J. Plant Physiol. 22:561-569.

Munns R, Tester M (2008). Mechanisms of salinity tolerance. Annu. Rev. Plant Biol. 59:651-681.

Ramesh SA, Tyerman SD, Xu B, Bose J, Kaur S, Conn V, Domingos P, Ullah S, Wege S, Shabala S, Feijó JA (2015). GABA signaling modulates plant growth by directly regulating the activity of plant-specific anion transporters. Nat. Commun. 6:7879.

Renault H, Roussel V, El Amrani A, Arzel M, Renault D, Bouchereau A, Deleu C (2010). The Arabidopsis *pop2-1* mutant reveals the involvement of GABA transaminase in salt stress tolerance. BMC Plant Biol. 10(1):20.

Rus A, Yokoi S, Sharkhuu A, Reddy M, Lee BH, Matsumoto TK, Koiwa H, Zhu JK, Bressan RA, Hasegawa PM (2001). AtHKT1 is a salt tolerance determinant that controls Na^+ entry into plant roots. Proc. Natl. Acad. Sci. USA 98:14150-14155.

Scholz SS, Reichelt M, Mekonnen DW, Ludewig F, Mithöfer A (2015). Insect herbivory-elicited GABA accumulation in plants is a wound-induced, direct, systemic and jasmonate-independent defense response. Front. Plant Sci. 6:01128.

Seo M, Koshiba T (2002). Complex regulation of ABA biosynthesis in plants. Trends Plant Sci. 7(1):41-48.

Seo M, Peeters AJ, Koiwai H, Oritani T, Marion-Poll A, Zeevaart JA, Koornneef M, Kamiya Y, Koshiba T (2000). The *Arabidopsis*

aldehyde oxidase 3 (AAO3) gene product catalyzes the final step in abscisic acid biosynthesis in leaves. Proc. Natl. Acad. Sci. USA 97:12908-12913.
Shelp BJ, Bown AW, McLean MD (1999). Metabolism and functions of gamma-aminobutyric acid. Trends Plant Sci. 4:446-452.
Shelp BJ, Mullen RT, Waller JC (2012). Compartmentation of GABA metabolism raises intriguing questions. Trends Plant Sci. 17:57-59.
Singh TN, Aspinal D, Paleg LG (1972). Proline accumulation and varietal adaptability to drought in barley: potential metabolic measure of drought resistance. Nat. New Biol. 236:188-190.
Studart-Guimarães C, Fait A, Nunes-Nesi A, Carrari F, Usadel B, Fernie AR (2007). Reduced expression of succinyl- coenzyme A ligase can be compensated for by up-regulation of the γ-aminobutyrate shunt in illuminated tomato leaves. Plant Physiol. 145:626-639.
Szekely G, Ábrahám E, Cséplő Á, Rigó G, Zsigmond L, Csiszár J, Ayaydin F, Strizhov N, Jásik J, Schmelzer E, Koncz C (2008). Duplicated P5CS genes of Arabidopsis play distinct roles in stress regulation and developmental control of proline biosynthesis. Plant J. 53:11-28.
Urano K, Maruyama K, Ogata Y, Morishita Y, Takeda M, Sakurai N, Suzuki H, Saito K, Shibata D, Kobayashi M, Yamaguchi-Shinozaki K (2009). Characterization of the ABA-regulated global responses to dehydration in Arabidopsis by metabolomics. Plant J. 57:1065-1078.
Wang Y, Gu W, Meng Y, Xie T, Li L, Li J, Wei S (2017). γ-Aminobutyric Acid Imparts Partial Protection from Salt Stress Injury to Maize Seedlings by Improving Photosynthesis and Upregulating Osmoprotectants and Antioxidants. Sci. Rep. 7:43609.
Xiong L, Zhu JK (2003). Regulation of Abscisic acid biosynthesis. Plant Physiol. 133:29-36.
Zhu J, Fu X, Koo D, Zhu JK (2007). An Enhancer Mutant of Arabidopsis salt overly sensitive 3 Mediates both Ion Homeostasis and the Oxidative Stress Response. Mol. Cell. Biol. 27(14):5214-5224.
Zhu JK (2002). Salt and drought stress signal transduction in plants. Annu. Rev. Plant Biol. 53:247-273.
Zik M, Arazi T, Snedden WA, Fromm H (1998). Two isoforms of glutamate decarboxylase in Arabidopsis are regulated by calcium/calmodulin and differ in organ distribution. Plant Mol. Biol. 37:967-975.

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