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African Journal of
Plant Science

December 2018
ISSN 1996-0824
DOI: 10.5897/AJPS
www.academicjournals.org

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African Journal of Plant Science

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Review

Review on effect of population density and tuber size on yield components and yield of potato (*Solanum tuberosum* L.)

Semira Nasir^{1*} and Bikila Akassa²

¹Department of Plant Science, Madda Walabu University College of Agriculture and Natural Resource, P. O. Box 247, Bale Robe Ethiopia.

²Bako Agricultural Research Center Western. Ethiopia.

Received 2 August, 2018; Accepted 26 October, 2018

Population density and seed tuber is an important agronomic management practices in the production of potato (*Solanum tuberosum* L.). However, potato farmers in Ethiopia often use random population density and tuber sizes, which contribute to the low yield of the crop. Thus the objective of this study is to review effect of population density and tuber size on yield components and yield of potato. The result of this review shows that the highest plant height and main stems number was found in wider spacing and in medium to large tuber size. In the same way, the number of tuber per plant, average tuber weight and marketable yield increased when potatoes are planted in medium intra row spacing (60 x 30) and using medium (35 to 45 cm) to large (45 to 55 cm) tuber size. However, total tuber number/ha and unmarketable yield increased in closer spacing and medium (35 to 45 cm) tuber size. Thus according to the review to increase total yield and marketable yield, it is better to use medium plant spacing (60 x 30 cm) and medium-sized tubers (35 to 45 mm).

Key words: Population density, potato growth, potato yield, tuber size.

INTRODUCTION

Potato (*Solanum tuberosum* L.) is one of the most widely grown tuber crops in the world and contributes immensely to human nutrition and food security (Miguel, 1985; Steven, 1999; Karim et al., 2010). It is the most important vegetable crop, constituting the fourth most important food crop in the world (Mattoo, 2006; Douches, 2013). Potato is one of mankind's most valuable food

crops and mainstay in the diets of people in many parts of the world (Struik and Wiersema, 1999). Potato is one of the economically most important tuber crops in Ethiopia that play key roles as a source of food and cash income for small farmer holders; and it is endowed with suitable climatic and endemic conditions for potato production. However, the national average yield is very

*Corresponding author. E-mail: seena.nasir7@gmail.com.

low (8.2 t ha^{-1}) compared to the world's average production (17.67 t ha^{-1}) (FAO, 2010). The major production problems that account for such low yield are unavailability and high cost of tuber seeds, lack of well adapted cultivars, poor agronomic practices, diseases, insect pests, inadequate storage, transportation and marketing facilities (Tekalign, 2005).

Tuber size and population density is one of the most important constraints limiting potato production in Ethiopia. Larger seed and closer spacing up to a certain limit increase the yield of tubers per unit area. The yield increases with a decrease in spacing (Banarjee et al., 1988). However, the optimal planting density differs depending on the environmental conditions, cultivars and soil fertility. On the other hand, planting large seed tubers is advantageous under certain circumstances such as unfavorable soil and weather conditions. As plant density increases, there is a marked decrease in plant size and yield per plant. This effect is due to increased inter-plant competition for water, light and nutrients. Therefore the objective of this review is to assess the effect of potato tubers size and population density on yield component and yield of potato.

EFFECT OF PLANT POPULATION AND TUBER SIZE ON PLANT HEIGHT

Plants grown at wider plant spacing of $75 \times 30 \text{ cm}$ had the highest plant height (77.48 cm) higher than the heights of plants grown at the spacing of $60 \times 20 \text{ cm}$, $50 \times 30 \text{ cm}$, and $50 \times 20 \text{ cm}$ by about 8.33, 8.4 and 15.47%, respectively (Zebenay, 2015). According to Bikila et al. (2014) the tallest plant height was observed when the inter row spacing and intra row spacing increase from 60×20 to 80×40 respectively. This may be due to better availability of nutrients, water and sun light since plants in wider spacing have less competition and grow more shoot; however, densely populated plants show intensive competition which leads to decrease in plant height. Wider intra row spacing resulted in reduction in plant height and in closer inter row spacing the highest plant height was observed. This is due to the presence of higher competition for sunlight among plants grown at closer intra row spacing (Tesfaye et al., 2013). Similar results obtain by Ashwani et al. (2013) showed that planting at wider intra row spacing resulted in reduction in plant height. In general, the plant height of the potato crop increases when plants are planted in closer intra row spacing due to competition for sunlight. In other words, when plants are planted in wider intra row spacing the same result may be obtained due to the plant getting enough mineral, water and sunlight.

Regarding the tuber size, the maximum plant height (75.39 cm) was obtained from large seed tuber sizes ($>56 \text{ mm}$) whereas shorter plant height was obtained

from small seed tuber size. Large seed tuber sizes ($>56 \text{ mm}$) produced the tallest plants which was higher than medium (35 to 45 mm) and small (25 to 34 mm) seed tuber sizes by about 4.9 and 8.9%, respectively. The variation in plant height might be due to the higher food reserves in tubers with larger size than small and medium seed tuber sizes (25 to 34 and 35 to 45 mm) which enhanced vegetative growth of the plant including the height of the plant (Zebenay, 2015).

EFFECT OF PLANT POPULATION AND TUBER SIZE ON MAIN STEM NUMBER

Planting densities of 4.17, 4.44, and 5.56 resulted in significantly highest number of main stems than the planting densities of 6.67 and $8.00 \text{ plant m}^{-2}$ (Alemayehu et al., 2015). In other findings, numbers of main stems were not influenced by plant population (Beukema and Van, 1990). However, stem number increased as a result of either by planting smaller tuber size or more tuber number per unit area per plant (Sturz et al., 2003). Young seed has a few sprouts that emerge slowly and hence produces a few main stems (Pavek and Thornton, 2009). The presence of internal inhibition limits sprouting at both the distal and dorsal ends of young seed tubers. Large size seed tubers produce more stems than small ones.

Number of stems produced by seed tubers planted at $60 \times 30 \text{ cm}$ spacing was significantly higher at a spacing of $60 \times 20 \text{ cm}$ (Rajadurai, 1994). The seed tuber size had significant ($P < 0.01$) influence on number of main stem per hill. Plants grown from large seed tuber size produced higher number of main stems per hill than other seed tuber sizes. The highest number of main stems per hill (5.69) was recorded from large seed tuber size which significantly exceeded that of 46 to 55, 35 to 45 and 25 to 34 mm seed tuber sizes by about 40.84, 57.62 and 82.37%, respectively. Plants grown from large seed tuber size ($>56 \text{ mm}$) produced highest number of stems per hill. The seed tuber size affects the growth of the crop due to large tubers having more number of buds. Higher number of bud per tuber produces more number of main stems (Zebenay, 2015).

EFFECT OF PLANT POPULATION AND TUBER SIZE ON TUBER NUMBER PER PLANT

The highest number of tuber per plant (10.93) was recorded at the wider intra row spacing 40 cm whereas the lowest number of tuber per plant (6.7) was obtained at closer spacing 10 cm (Table 1). This is because in wider intra row spacing there is minimum competition among plants for space and resource and better exposure for light; this results in increased number of tuber per plant (Tesfaye et al., 2013). Number of tuber

Table 1. Means for tuber number, total yield, marketable yield and unmarketable yield as affected by intra row spacing.

Treatment	Tuber number (count hill ⁻¹)	Total tuber yield (t ha ⁻¹)	Marketable tuber yield (t ha ⁻¹)	Unmarketable tuber yield (t ha ⁻¹)
Intra row spacing (cm)				
10	6.70	34.43	18.27	16.16
20	8.43	31.49	21.71	9.74
30	10.56	30	23.54	6.46
40	10.93	26.09	21.19	4.89

Source: Tesfaye et al. (2012).

per plant increases with increasing seed tuber size and planting space. Large size seed tuber produces significantly more number of tubers over small. Tuber number per plot increases with increasing seed tuber size. However, the difference in tuber numbers between small and medium size seed tubers was not significant (Rajadurai, 1994).

Plants grown from large seed tuber size produced high total tuber number per hill whereas small seed tuber size produced low total tuber numbers per hill. Large seed tuber size (>56 mm) significantly exceeded in producing total number of tubers than that of 46-55, 35- 45 and 25-34 mm by about 12.47, 20.55 and 26.30%, respectively. Total tuber number per hill produced from 46-55 mm seed tuber size is not statistically and significantly different with 35-45 mm seed tuber size. Plants grown at closer plant spacing of 50 x 20 cm produced highest total tuber number per hill higher than plants spaced at 60 x 20 and 75 x 30 cm by about 11.27 and 12.18 %, respectively. However, total tuber number per hill produced at 50 x 20 cm plant spacing has no statistically significant difference with 60 x 30 cm and 50 x 30 cm plant spacing. The production of total number of tubers per hill increased as plants grown at narrow plant spacing and decreased at wider plant spacing. This might be due to the higher number of plants produced at closer plant spacing than plants at wider spacing which led to the production of highest number of total tubers per hill (Zebenay, 2015). Tuber numbers were significantly affected by plant population density, with the highest density plants having a lower number of tubers per plant (Michael et al., 2011).

EFFECT OF PLANT POPULATION AND TUBER SIZE ON AVERAGE TUBER WEIGHT (G)

Maximum average tuber weight (119.61 g) was recorded for plants grown from medium seed tuber size (35 to 45 mm) and planted at wider plant spacing (75 x 30 cm); the lowest average tuber weight (55.91 g) was obtained at closer plant spacing (50 x 20 cm) and large seed tuber size (>56 mm). Plants grown from medium seed tuber

size (35 to 45 mm) across all plant spacing had maximum average tuber weight than other seed tuber sizes. Plants at wider spacing grown from medium seed tuber sizes gave maximum tuber weight than plants grown at other plant spacing and from seed tuber sizes. When plant density increased the weight of tubers decreased in all seed tuber sizes except in plants grown at plant spacing of 50 x 20 cm and from small seed tuber size (25 to 34 mm). The production of tubers with higher weight when medium seed tuber size (35 to 45 mm) was used as planting material with wider space (75 x 30 cm) might be due to the production of optimum number of stems with lesser competition for resource between plants compared to small and large seed tuber sizes planted at closer plant spacing (Zebenay, 2015).

EFFECT OF PLANT POPULATION AND TUBER SIZE ON TOTAL TUBER YIELD /HA

Increasing the planting density from 4.44 to 8.00 plants m⁻² significantly increased total tuber number/ha. The highest tuber yield per hectare was obtained at closer spacing of 10 cm whereas the lowest was obtained at wider intra row spacing of 40 cm. The wider intra row spacing yield per hectare was reduced due to the insufficient number of plant grown per hectare compared to plant grown at closer intra row spacing per hectare. The maximum yield was obtained at closer plant spacing than wider plant spacing. This might be attributed to efficient use of available soil nutrients and other growth factors in plants grown at closer plant spacing than wider plant spacing. The increased yield at higher densities might be due to the ground being covered with green leaves earlier (earlier in the season, light is intercepted and used for assimilation), fewer lateral branches being formed and tuber growth starting earlier (Zebenay, 2015).

As shown in Table 2, narrow spacing increases the hectare yield and decreases the yield per plant. The highest yield was obtained with large size seed tuber (45-55 cm) planted in narrow spacing (60 x 20cm). However, the combination of large size seed tuber and narrow spacing produce many small side size tubers of low

Table 2. Effect of seed tuber size and planting space on tuber yield /ha.

Tuber size	Planting space		
	60x20 cm	60x30 cm	60x50 cm
15-30 mm	24.43	20.89	17.20
30-45 mm	24.22	24.89	20.88
45-55	28.45	24.37	21.23

Source: Rajadurai (1994).

market value (Rajadurai, 1994).

The highest yield was obtained from 65 cm inter row spacing; whereas the lowest yield was recorded at 80 cm inter row spacing. Regarding the intra row spacing the higher total yield per hectare was obtained from 20 cm intra row spacing. As intra row spacing increased from 20-35 cm, total tuber yield decreased from 37.54 to 29.38 t/ha. Intra -row spacing of 35 cm showed lower total tuber yield. It was clearly evident from the result that the yield of seed tuber per hectare increased with decreasing plant spacing. The increased yield was attributed to more tubers produced at the higher plant population per hectare although average tuber size decreased because of increasing inter plant competition at closely spaced plants leading to more unmarketable tuber yield. At closer spacing, there is high number of plant per unit area which brings about an increased ground cover that enables more light interception, consequently influencing photosynthesis (Harnet et al., 2014). Yield performance (kg/ha) was greatest at the medium density level (90 by 30 cm), followed by plants established at 90 by 45 cm. Reducing the intra-row spacing from 45 to 30 cm significantly ($p < 0.05$) increased plant population and subsequently increased the yield (kg/ha) performance. Tuber yield was significantly ($p < 0.05$) affected by plant density as plants planted at 90 by 30 cm exhibited highest yield performance compared to those planted at 90 by 15 cm and 90 by 45 cm (Michael et al., 2012).

EFFECT OF PLANT POPULATION AND TUBER SIZE ON MARKETABLE TUBER YIELD /HA

Effect of row spacing and seed type on yield found that those plants grown from large seed pieces produced higher marketable yield at the widest spacing (Robert et al., 2015). The highest marketable tuber yield was obtained in response to planting the tubers at the spacing of 60 x 30 cm whereas the lowest marketable tuber yield was recorded at the spacing of 50 x 30 cm plant spacing. Plant spacing of 60 x 30 cm produced higher marketable yield than 50 x 30 cm and 75 x 30 cm plant spacing by about 12.04 and 9.53%, respectively. Similarly, marketable tuber yield produced at 60 x 20 cm and 50 x 20 cm exceeded that of 50 x 30 cm plant spacing by

about 8.65 and 8.72%, respectively. Plant spacing of 60 x 30 cm, 60 x 20 cm and 50 x 20 cm produced marketable tuber yield per hectare without significant difference (Zebenay, 2015).

The highest marketable yield was obtained at the wider intra row spacing of 30 cm whereas the lowest was obtained at closer spacing of 10 cm. At wider intra row spacing due to presence of minimum competition, plants absorbed the sufficient available resource and intercepted more light. This increased their photosynthesis efficiency for higher photo assimilation production and ultimately resulted in increased more marketable tuber yield (Tesfaye et al., 2013). According to Alemayew et al. (2015) increasing the planting density from 4.44 to 6.67 plants m^{-2} significantly increased total and marketable tuber yield by 5.21 and 4.67 t/ha.

EFFECT OF PLANT POPULATION AND TUBER SIZE ON UNMARKETABLE TUBER YIELD /HA

The highest unmarketable yield was obtained at the closer intra row spacing of 10 cm whereas the lowest was obtained at closer spacing of 40 cm (Table 1). This is due to presence of higher competition between plants in closer intra row space (Tesfaye et al., 2013). The highest unmarketable tuber yield was obtained at closer plant spacing (50 x 20 cm) whereas the lowest unmarketable tuber yield was recorded at wider plant spacing (75 x 30 cm). the closer spacing of 60 x 20 cm and 50 x 30 cm would need more seed tubers than the spacing of 60 x 30 cm, the latter spacing (60 x 30 cm) would be more profitable. The highest unmarketable tuber yield was produced at the highest planting density of 8.00 plants m^{-2} , and exceeded the unmarketable tuber yield obtained at the lowest planting density of 4.17 plants m^{-2} by 0.863 t/ha (Alemayew et al., 2015). Generally, plants grown at closer spacing produced high unmarketable tuber yield than plants grown at wider plant spacing. Increasing plant density also increased the yield of unmarketable tuber yield. Closer plant spacing increased competition of plants for growth factors due to high number plant per unit area than wider plant spacing which led to producing high number of under size tubers which was high unmarketable tuber yield (Zebenay, 2015).

CONCLUSION

This review revealed that plant spacing of 60 x 30 cm and medium (35 to 45 mm) to large (45 to 55mm) seed tuber sizes resulted in the production of higher marketable tuber yields and higher tuber yield/ha. Even if using medium (35 to 45 mm) to large (45-55 mm) seed tuber sizes resulted in higher production, considering the income of the user, using medium seed tuber sizes (35 to 45 mm) with plant spacing of 60 x 30 cm is appropriate.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Full Length Research Paper

Effect of genotype and environment on grain quality of sorghum (*Sorghum bicolor* L. Moench) lines evaluated in Kenya

Njuguna V. W.^{1*}, Cheruiyot E. K.¹, Mwonga S.¹ and Rono J. K.²

¹Department of Crops, Horticulture and Soil, Egerton University, P. O. Box 536-20115, Njoro, Kenya.

²Department of Biochemistry and Molecular Biology, Egerton University, P. O. Box 536-20115, Njoro, Kenya.

Received 2 February, 2018; Accepted 15 March, 2018

Grain sorghum (*Sorghum bicolor*) has a great potential for use as food and beverage in developing countries. However, information regarding the effect of the agro-ecological environments on the grain quality attributes of selected sorghum lines desirable for malting and brewing and for baking in Kenya, is lacking. The experiments of this study were conducted at different environmental locations in Kisumu, Siaya and Busia Counties of Kenya. Nine sorghum lines were sown in plots in Randomized Complete Block Design (RCBD) and replicated three times. Panicles from two central rows of each plot were harvested at physiological maturity to provide grains that were used for proximate analysis. The percentage crude protein, tannin and starch content were determined. The amount of starch varied with sorghum lines and growing environments, ranging between 29.7 and 80.2%. SDSAI × ICSR43 line recorded tannin content in the range of 8.00 to 24.33 mg/100 ml tannic acid equivalents. Crude protein content and starch ranged 8.9 to 15.4% and 29.7 to 80.2%, respectively across environments. The combined analysis showed that the growing environment variously affected the nutritional and anti-nutritional content of sorghum lines. This implies that breeders should consider stability of the quality parameters that define commercial utilization of these sorghum lines.

Key words: Sorghum lines, lower midland zone (LM), starch, crude protein, tannins.

INTRODUCTION

Grain sorghum (*Sorghum bicolor* L. Moench) has considerable potential for use as a human food and as raw material for lager and stout beer, and in baking. Commercial processing of sorghum grains into value-added food and beverage products is an important driver for economic development in the developing countries

(Taylor et al., 2004). In malting and brewing, the quality aspect of sorghum grains that is important includes amylose, amylopectin, starch, protein, and tannin contents. Each of these quality attributes play a considerable role in the quality of beer obtained after brewing (Schnitzenbaumer and Arendt, 2014).

*Corresponding author. E-mail: vwanjiku89@yahoo.com.

Sorghum grown for food has been reported to have health benefits protecting against colon cancer due to presence of antioxidants (Darvin et al., 2015). Sorghum grains are further preferred for fighting obesity due to its high tannin content that lowers digestibility. The growing demand for sorghum globally is attributed to its diversified uses including food products, malted beverages and ethanol production (Oyediran et al., 2017). Recent studies have shown that sorghum can be fortified with legumes to create cereal-based diets rich in protein (Okoye et al., 2017). Wheat flour has been the most important ingredient of bread for many years; however, sorghum is gaining popularity as an alternative for wheat in bread making. The composite flour made from sorghum for baking biscuits has high nutritional value (Rao et al., 2018).

The quality of sorghum grain is affected by factors such as genotype, climate, soil type, and fertilizer supply, among others; which can affect its chemical composition and the nutritive value (Ebadi et al., 2005). Johnson (2005), working on the influence of corn and sorghum characteristics on wet milling and nixtamalization, found that high temperatures and water stress result in lower starch concentrations. Wallwork et al. (1998) indicated that if a short period of high temperature occurs at a certain point in the grain filling period, it may affect one or more components, which are being synthesized concurrently, resulting in a different composition of the mature grain. High temperatures and moisture stress can limit the amount of grain fill operating through the metabolism of starch in the grain. Bleidere and Sterne (2008) working on spring barley reported that hot and dry conditions occurring during the cell division period in starchy endosperm resulted in shortening the cell division period, thus influencing the accumulation of starch; hence, low starch and higher protein. This is because the accumulation of starch is more sensitive to high temperatures than to the accumulation of nitrogen, which frequently determines increases in the grain nitrogen proportion, and thus results in higher protein contents (Schelling et al., 2003).

The release of new sorghum lines suitable for malting and brewing, and for use in baking, is of great significance to emerging farmers in Kenya who would wish to venture into sorghum production for commercial purposes. Industrial sorghum has a ready market and is likely to provide farmers with better returns. However, information regarding the effect of the agro-ecological environments on the grain quality attributes of sorghum desirable for malting and brewing and for baking in Kenya, is lacking. The acquisition of good quality grain is fundamental to produce acceptable food and beverage products from sorghum. Thus, the study was carried out to determine the effect of agro-ecological environment on the grain quality of selected sorghum lines for industrial uses, in order to advise sorghum farmers on sustainable production of quality grains.

MATERIALS AND METHODS

Study location

Nine sorghum lines were grown at Masumbi and Sagam counties, both in the Lower Midland (LM) zone 1, in long and short rainy seasons, respectively, and at Mundika (LM 2) in both long and short rainy seasons in 2014. The short rainy season was between March and July while the long rainy season was experienced between September and December, 2014. Masumbi (00° 01' 73.0'' N, 034° 21' 87.4'' E), located in Maranda Division at an altitude of 1370 m above sea level, is in Lower Midland (LM 1) zone. Dominating soils are well-drained, moderately deep to very deep, dark red to strong brown, friable clay; and in many places shallow (Jaetzold et al., 2005). Mundika (00° 24' 56.6'' S, 034° 07' 93.1'' E) is in the LM 2 zone characterized by well-drained, shallow to moderately deep, yellowish red to dark redish brown, friable, gravely sandy clay to clay soils (Jaetzold et al., 2005). The rainfall variability in this subzone is high, and hence the reliability is low. Sagam (0° 03' 20.86'' N, 034° 32' 31.06'' E) is in LM 1 zone at 1387 m above sea level. Dominating soils are moderately well drained, moderately deep to deep, (very) dark brown, firm clay; in many places slightly calcareous and/or cracking clay; with a humic topsoil (Jaetzold et al., 2005). This zone receives an average annual rainfall of 1450 to 1650 mm where 60% reliability of the growing periods during the 1st and 2nd rainy seasons is more than 190 and 130 to 150 days, respectively (Jaetzold et al., 2005). In Sagam (LM 1), the annual temperature range is 21.2 to 22.8°C.

Experimental design and treatments

The experiment was set up in a Randomized Complete Block Design (RCBD) with nine experimental units each measuring 4 m by 2.5 m and replicated three times. A path of 1.5 m separated the replicates. Each experimental unit had four rows of a specific sorghum line. The treatments were the five sorghum lines suitable for baking and four suitable for malting and brewing, evaluated at the four agro-ecological environments. These lines are SDSAI X ICSR 43, IS 9203, IS25561, IS 25557, *Sima*, *Gadam*, *Serena*, *Siaya* # 2-3 and *Abaleshya*. *Sima*, *Gadam*, and *Serena* were used as the controls for the line identified for malting and brewing (SDSAI X ICSR 43), while *Siaya* #2-3 and *Abaleshya* were the checks for lines identified for use in baking (IS 9203, IS25561 and IS 25557).

Agronomic management

Land was disc ploughed and harrowed to fine tilt. Sorghum was sown at onset of rainy season at a seed rate of 8 kg ha⁻¹ on 18th March and 10th September, 2014 for first and the second season, respectively. The inter row spacing for the drills was 60 cm at a planting depth of 2.5 to 4 cm. Due to low nitrogen (0.11 to 0.17%) and phosphorous (5.5 to 9.8 ppm) levels in these soils, there was a uniform fertilizer application in all the plots. Nitrogen was applied using Calcium Ammonium Nitrate at the rate of 40 kg N ha⁻¹ split into two applications of 20 kg N ha⁻¹ at planting and top dressed with 20 kg N ha⁻¹. Phosphorous was added at planting using Triple Super Phosphate at a rate of 17.2 kg P₂O₅ ha⁻¹. Two weeding operations were carried out, with the first weeding being done at 2 to 3 weeks after seedling emergence. After the first weeding the crop was thinned to a spacing of 60 cm (inter row) by 10 cm (intra row). The second weeding was carried out when the crop was about 45 cm high. Harvesting was done when the crop had reached physiological maturity approximately 16 weeks after sowing for the first season and 14 weeks for the second season.

Sample collection

Harvesting of panicles from the two inner rows per plot was done at physiological maturity. It involved cutting the panicles at the collar of the top-most leaf using secateurs. Samples were put in well-labelled bags. Panicles were then sun dried for 2 weeks to moisture content of 13% followed by threshing and winnowing to obtain clean grains. The grains were thereafter milled finely to pass through a 1 mm sieve. The flour obtained was used for proximate analysis.

Proximate analysis

Determination of crude protein content

Total nitrogen and protein was determined using the Kjeldahl method (AOAC, 1999). One-tenth gram finely milled sorghum grains were weighed and transferred into a digestion tube. Selenium catalyst mixture weighing 1 g was mixed with the samples and 5 ml of 96% sulphuric acid was added into the tube. The tubes were then heated cautiously in the digester at the fume cupboard until the digest was clear. The sample was transferred to a 100 ml volumetric flask, and distilled water was added into a 100-ml graduated flask up to the mark. Boric acid indicator solution of 5 ml was then transferred to a 100-ml conical flask containing 5 drops of mixed indicator, and then placed under the condenser of the distillation apparatus. 10 ml of the clear supernatant liquid of the digest was then transferred into the apparatus, and 10 ml of 46% sodium hydroxide added and then rinsed again with distilled water. Distillation then commenced. After the first distillation, drops reached the boric acid indicator solution, and colour changed from pink to green. A total of 150 ml of the distillate was collected. The solution was titrated with 0.0174 N sulphuric acids until the colour changed from green to pink. The sample was replicated three times for each sorghum line. Total N was determined using the formula:

$$\% N = (a \times N \times Mw \times 100 / b \times c) \times 100$$

where a = ml of sulphuric acid used for titration of the sample, N = Normality of sulphuric acid (0.0174), Mw = Molecular weight of N₂ (0.014), c = ml digest taken for distillation (10 ml), b = g sample taken for analysis (0.1 g), and % Crude Protein = 6.25 × % N.

Determination of tannin content

Tannins content was determined through Folin-Denis method (Schanderl, 1970). Powdered flour (0.5 g) was weighed and transferred to a 250-ml conical flask followed by addition of 75 ml of water. The flask was heated gently and boiled for 30 min and then centrifuged at 2000 rotations per minute for 20 min. The supernatant was collected in a 100-ml volumetric flask. 1 ml of the sample extract was transferred to a 100-ml volumetric flask containing 75 ml water. 5 ml of folin reagent and 10 ml of 35% sodium carbonate solution were added and then diluted to 100 ml with water. The sample was shaken and the absorbance read at 700 nm after 30 min. A graph was prepared using 0 to 100 mg tannic acid, where 1 ml contained 100 mg tannic acid. The sample was replicated three times for each sorghum line. The tannin content of the sample was calculated as tannic acid equivalent from the standard curve.

Determination of starch

Percent starch content was determined by the Anthrone method (Hodge and Hofreiter, 1962), whereby 0.2 g of milled grain sample was homogenized in 80% hot ethanol to remove sugars. The

residue was then centrifuged and retained. The residue was dried well over a hot water bath. To the residue, 5.0 ml of distilled water and 6.5 ml of 52% perchloric acid was added and then extracted at 0°C for 20 min. The supernatants were centrifuged, pooled and made up to 100 ml. 0.1 ml of the supernatant was pipetted out and made up to the volume of 1 ml with distilled water. The standards were prepared by taking 0.2, 0.4, 0.6, 0.8 and 1 ml of the working standard solution and the volume made up to 1 ml in each tube with water. 4 ml of Anthrone reagent was then added to each tube and the sample heated for 8 min in a boiling water bath. Each sample was cooled rapidly and the intensity of green to dark green colour was read using a spectrophotometer at 630 nm. The sample was replicated three times for each sorghum line. The glucose content in the sample was determined using the standard calibration graph and then the value was multiplied by a factor of 0.9 to arrive at the starch content.

Data analysis

Data were subjected to analysis of variance using SAS version 8.1 (Littel et al., 2002). Means were separated according to Least Significant Difference (LSD) whenever the sorghum line effects were significant ($P \leq 0.05$).

RESULTS AND DISCUSSION

Crude proteins

There were significant differences in the crude protein content in the different sorghum lines. Results showed that the protein content of the sorghum lines evaluated ranged from 8.93 to 13.79, 10.32 to 15.35, 9.08 to 14.64, and 9.36 to 12.38% when grown in a long rainy season in Mundika (LM 2) and Masumbi (LM 1) and during short rainy season in LM 2 and Sagam (LM 1), respectively (Table 1). Some inbred and hybrid lines of sorghum in Kansas (Hicks et al., 2002) and African sorghum lines (Aba et al., 2005) had a similar crude protein range of 10.3 to 16.5% and 10 to 16.45%, respectively. Genetic variability of sorghum accessions was reported to contribute to the variation in crude protein content of the accessions evaluated (Chavan et al., 2009; Ng'uni et al., 2012).

In this study, the growing environment had an affect on accumulation of crude protein in some of the sorghum lines evaluated (Table 1). Among the lines suitable for malting, SDSAI × ICSR43 line maintained statistically similar amounts of crude protein in all the test environments, whereas *Gadam* and *Serena* showed a drop in crude protein when cultivated in Sagam (LM 1). *Sima*, in contrast, yielded higher crude protein when grown in Masumbi (LM 1) and in the long rainy season in LM 2, while a low crude protein content was produced upon growing the line during short rainy season in LM 2 and in Sagam (LM 1).

Among the lines suitable for baking, lines IS 25557 and Siaya #2-3 were the only two lines that showed interaction with the environment in which they were cultivated in terms of crude protein content (Table 1). IS 25557 line had low crude protein when grown in Sagam

Table 1. Crude protein levels of the nine sorghum lines evaluated at Mundika (LM 2) during long (LR) and short rain (SR) season, Masumbi (LM 1) and Sagam (LM 1).

Sorghum line	% Crude Protein across and within environments				LSD _{0.05}
	Mundika (LM 2)	Masumbi (LM1)	Mundika (LM2)	Sagam (LM1)	
	Long rains		Short rains		
SDSAI × ICSR43	11.92 ^{†ab} _{A†}	11.62 ^b _A	10.32 ^{cd} _A	12.06 ^a _A	6.67
Sima	9.08 ^b _{AB}	15.35 ^a _A	12.75 ^{ab} _B	10.74 ^{ab} _B	4.57
Serena	12.60 ^{ab} _{AB}	12.74 ^{ab} _A	10.84 ^{bcd} _A	9.84 ^{bc} _B	2.18
Gadam	13.81 ^{ab} _A	13.44 ^{ab} _A	12.56 ^{abc} _A	8.25 ^c _B	1.94
IS 9203	14.64 ^a _A	10.52 ^b _A	11.37 ^{bc} _A	10.74 ^{ab} _A	4.37
IS 25561	10.17 ^{ab} _A	10.48 ^b _A	10.62 ^{bcd} _A	9.36 ^{bc} _A	2.86
IS25557	12.31 ^{ab} _A	11.90 ^{ab} _{AB}	13.79 ^a _{AB}	9.73 ^{bc} _B	3.23
Siaya #2-3	14.59 ^a _C	10.32 ^b _{BC}	8.93 ^d _A	12.38 ^a _B	2.75
Abaleshya	13.61 ^{ab} _A	11.21 ^b _A	10.51 ^{bcd} _A	11.06 ^{ab} _A	4.24
LSD _{0.05}	4.82	3.47	2.31	1.99	

[†]Means followed by subscript same capital letter ACROSS the row do not significantly differ ($p > 0.05$). ^{*}Means followed by superscript same small letter DOWN the column do not differ significantly ($p > 0.05$).

(LM 1), while Siaya #2-3 yielded high crude protein when cultivated in LM 2 during the short rainy season, moderately high crude protein when grown in Masumbi (LM 1) and lowest amount was obtained when the line was grown in LM 2 during the long rainy season. Protein content and composition vary due to genotype and water availability, temperature, soil fertility and environmental conditions during grain development (Hulse et al., 1980; Ebadi et al., 2005). The protein content of sorghum variety is important if the variety is to be designated as grain sorghum for malting and brewing purposes (FAO, 1995; Beta et al., 1995). This is because proteins degradation by proteolytic enzymes to peptides and amino acids (Jones, 2005a, b) provides energy for the yeasts during the fermentation process leading to production of alcohol. Kiprotich et al. (2014) reported that desirable protein contents of sorghum for malting and brewing should be within the range of 5 to 10%. SDSA1 × ICSR43 line recorded 10.32% crude protein when grown in LM 1 during the long rain season, which was not significantly different from amount of crude protein yielded in the rest of the test environments. This shows that the new variety SDSA1 × ICSR43 can be adopted by breweries due to its superior qualities in terms of yield and quality attributes.

Tannins

The tannin content of the sorghum lines ranged between 8.00 and 70.00 mg/100 ml tannic acid equivalents (Table 2). This is consistent with Kiprotich et al. (2014) who reported similar ranges of 6.88 to 79.89 mg/100 ml tannic acid among locally grown sorghum genotypes in Kenya. The SDSA1 × ICSR43 line had a tannin content that ranged between 8.00 to 24.33 mg/100 ml tannic acid

equivalents depending on the growing environment. Relative to the check lines, the tannin content of SDSA1 × ICSR43 line was 45.27% lower than for *Sima* when grown in Mundika (LM 2) during the short rainy season. Growing the lines in Masumbi (LM 1), SDSA1 × ICSR43 line had 39.56, 46.27 and 54.32% less tannin than *Sima*, *Gadam* and *Serena*, respectively. During the long rainy season in LM 2, lowest amounts of tannins were obtained for SDSA1 × ICSR43 than the check lines; while planting these lines in Sagam (LM 1) their tannin content was statistically analogous.

Sorghum lines suitable for baking had tannin levels ranging from 41.00 to 70.00 mg/100 ml tannic acid equivalents (Table 2). No significant statistical variation in tannin content was observed among the sorghum lines when grown in LM 2 during the short rainy season and in Sagam (LM 1). However, evaluating the lines during the long rainy season in LM 2, Siaya #2-3 recorded the highest amount of tannins, while IS 25557 had the lowest amount of tannins relative to other lines when evaluated at Masumbi (LM 1). High tannin sorghums are desirable in making food products due to their palatability (Awika et al., 2004). Good quality breads containing tannin sorghum bran have high antioxidant and dietary fiber levels, with a natural dark brown colour and excellent whole grain flavor (Gordon, 2001). In addition, healthy bread mixes containing tannin sorghum bran, barley flour, and flax seed have been developed (Rudiger, 2003).

The combined analysis showed that the agro-ecological environment was the main source of variation in tannin content of sorghum lines, particularly SDSA1 × ICSR43, IS 25557, *Abaleshya* and *Sima* (Table 2). Trikoesoemaningtyas et al. (2015) reported similar findings on sorghum lines evaluated in Indonesia. Taleon et al. (2012) on evaluating black sorghum found that the total flavonoid content was affected strongly by

Table 2. Tannin levels of the nine sorghum lines evaluated at Mundika (LM 2) during long (LR) and short rain (SR) season, Masumbi (LM 1) and Sagam (LM 1).

Sorghum line	Tannin (mg/100ml) across and within environments				LSD _{0.05}
	Mundika (LM 2)	Masumbi (LM1)	Mundika (LM2)	Sagam (LM1)	
	Long rains		Short rains		
SDSAI × ICSR43	9.67 [‡] _B †	9.67 ^d _B	8.00 ^f _B	24.33 ^c _A	5.15
Sima	25.67 ^b _B	22.33 ^c _B	17.67 ^e _B	53.00 ^{abc} _A	12.23
Serena	16.67 ^{bc} _A	32.67 ^b _A	29.67 ^d _A	31.33 ^{bc} _A	21.36
Gadam	15.67 ^{bc} _A	26.33 ^{bc} _A	18.33 ^e _A	41.33 ^{abc} _A	26.84
IS 9203	46.00 ^a _A	50.33 ^a _A	49.67 ^b _A	46.67 ^{abc} _A	11.52
IS 25561	45.00 ^a _A	51.00 ^a _A	47.33 ^b _A	54.33 ^{abc} _A	25.72
IS25557	41.00 ^a _{AB}	32.00 ^b _C	46.00 ^{bc} _{BC}	53.33 ^{abc} _A	11.65
Abaleshya	47.67 ^a _B	49.67 ^a _B	41.00 ^c _B	70.00 ^a _A	20.11
Siaya #2-3	25.67 ^a _A	52.00 ^a _A	58.67 ^a _A	60.33 ^{ab} _A	26.09
LSD _{0.05}	13.225	9.090	5.833	35.350	

†Means followed by subscript same capital letter ACROSS the row do not significantly differ ($p > 0.05$). ‡Means followed by superscript same small letter DOWN the column do not differ significantly ($p > 0.05$)

environment, mainly due to the differential effect of abiotic factors such as light and temperature, and differential intensity of fungal infection. Wu et al. (2016) indicated that tannin biosynthesis in the sorghum grains might be inhibited under the higher growing temperature. However, in this study all sorghum lines evaluated recorded higher amount of tannins when grown in an environment that received the highest maximum daily temperature of 28°C, among the test environments. Similar findings were reported by Wang and Zheng (2001) working on strawberry where the individual phenolics of strawberry increased when growth temperature increased from 18/12 to 30/22°C (day/night). Kiprotich et al. (2014) recommended that sorghum grains suitable for malting and brewing should not have tannin levels greater than 18.13 mg/100 ml since high tannin levels poses a challenge during the brewing process. Tannins inhibit the activity of alpha amylase (Alonso et al., 2000), and this lowers hydrolysis of starch that is essential for brewing. This study has shown that growing SDSAI × ICSR43 in LM 1 (Sagam) during the short rainy season causes a drastic increase in tannin content for the line, thus affecting its suitability for use in brewing. Cultivation of SDSAI × ICSR43 in LM 1 and LM 2 during the long rainy season and LM 2 during the short rainy season produces quality grains for malting and brewing with regard to tannin levels. This suggests it requires about 846.4 mm of rainfall during the crop growing period and an average minimum and maximum temperature of 20 and 28°C to obtain the optimum grain quality for malting and brewing.

Starch

The sorghum lines evaluated had starch content ranging

from 29.73 to 80.23% (Table 3). Generally, the malting and brewing sorghum lines had relatively higher amounts of starch compared to the lines suitable for baking. This confirms the findings by Almodares and Sepahi (1996) that the cultivar of sorghum affects the levels of sorghum non-structural carbohydrates. SDSAI × ICSR43 line yielded similar amounts of starch as the check lines in all the test environments. On the other hand, an environmental effect on starch content was noted on lines SDSAI × ICSR43, *Sima* and *Serena*, while *Gadam* maintained a comparable amount of starch in all the test environments. Sorghum grain starch accumulation is subject to environmental factors since it is a quantitatively inherited trait (Bing et al., 2014). The SDSAI × ICSR43 line yielded the highest amount of starch when grown in Mundika (LM 2), both in long and short rainy seasons, moderate amount in Masumbi, and lowest amount when grown in Sagam. Lines *Sima* and *Serena* when grown in Sagam (LM 1) and Masumbi (LM 1), respectively, produced lowest amount of starch.

All the lines suitable for baking did not differ in the amount of starch within each growing environment (Table 3). In addition, genotype by environment effect was only observed with IS 25561 line whereby in Mundika (LM 2) during the short rainy season the highest starch content was realized; whereas in the other test environments lower amounts of starch were recorded that were statistically similar across the environments. Many researchers have reported the effect of growing environment on the chemical composition of sorghum grain and other cereals (Beta and Corke, 2001; Tester and Karkalas, 2001; Matsuki et al., 2003; Kiprotich et al., 2014; Trikoesoemaningtyas et al., 2015). However, the current study has shown that variation in starch content among sorghum lines suitable for use in baking was rather based on genotype than the growing environment.

Table 3. Starch content of the nine sorghum lines evaluated at Mundika (LM 2) during long (LR) and short rain (SR) season, Masumbi (LM 1) and Sagam (LM 1).

Sorghum line	% Starch across and within environments				LSD _{0.05}
	Mundika (SR,LM 2)	Sagam (LM1)	Mundika (LR,LM2)	Masumbi (LM1)	
	short rains		long rains		
SDSAI × ICSR43	71.20 ^{abc} _{A†}	41.43 ^b _{BC}	79.35 ^a _{AB}	56.43 ^{abc} _C	17.81
Sima	72.77 ^{abc} _A	29.73 ^{ab} _{AB}	78.80 ^a _{AB}	71.40 ^a _B	23.78
SERENA	81.67 ^a _{AB}	49.10 ^{ab} _B	56.77 ^{ab} _A	44.08 ^c _B	28.90
GADAM	80.23 ^{ab} _A	60.33 ^a _A	62.53 ^{ab} _A	64.63 ^{ab} _A	20.80
IS 9203	55.30 ^d _A	56.57 ^{ab} _A	64.93 ^{ab} _A	55.07 ^{bc} _A	24.63
IS 25561	68.33 ^{abcd} _B	41.67 ^b _B	51.33 ^b _A	52.67 ^{bc} _B	12.40
IS25557	61.50 ^{dc} _A	54.03 ^{ab} _A	69.91 ^{ab} _A	58.10 ^{abc} _A	27.94
Siaya #2-3	64.87 ^{bcd} _A	56.90 ^{ab} _A	58.86 ^{ab} _A	50.13 ^{bc} _A	18.21
Abaleshya	64.93 ^{bcd} _A	51.63 ^{ab} _A	62.17 ^{ab} _A	47.43 ^c _A	22.95
LSD _{0.05}	15.51	17.39	25.81	15.74	

†Means followed by subscript same capital letter ACROSS the row do not significantly differ ($p>0.05$). ‡Means followed by superscript same small letter DOWN the column do not differ significantly ($p>0.05$).

Similar findings on genetic variations in starch content were reported in triticale (*× Triticosecale*) (Burešová et al., 2010) and in wheat (*Triticum aestivum* L.) (Labuschagne et al., 2007; Massaux et al., 2008).

Conclusion

The combined analysis showed that growing environment affected the nutritional and anti-nutritional content of sorghum lines with different magnitudes. The variances due to sorghum lines were higher for starch content and protein, but the variability observed for tannin content were mostly due to the agro-ecological environment of cultivation. The presence of genotypes × environmental interaction resulted in differential nutritional values of sorghum grains over environments. The results indicated that while conducting yield stability trials, breeders should not only focus on agronomic characters and yield potential, but should also consider stability of the quality parameters that define commercial utilization of these sorghum lines.

This study, therefore, recommends that during the long rainy season, lower midland zone (LM) 1, is ideal for the cultivation of line SDSA1 × ICSR43. This zone produces grains of required quality in terms of their usage in malting and brewing. Lower midland zone 2 is a stable environment for cultivation of the sorghum lines evaluated.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

ACKNOWLEDGEMENTS

The authors acknowledge Kenya Agricultural Productivity

and Agri-business Programme for funding the research. The Department of Crops, Horticulture and Soil Sciences in Egerton University is also thanked for providing facilities that ensured smooth running of the project.

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Full Length Research Paper

Nitrogen release dynamics of *Erythrina abyssinica* and *Erythrina brucei* litters as influenced by their biochemical composition

Abebe Abay

Central Ethiopian Environment and Forest Research Centre, P. O. Box 31037, Addis Ababa, Ethiopia.

Received 19 September, 2018; Accepted 1 November, 2018

Litter mineralization is a crucial process in providing nutrients through decomposition to plants, which also depends in the biochemical composition of the litter and soil properties as well. Decomposition rate of *Erythrina abyssinica* and *Erythrina brucei* in Luvisol was investigated in relation to their nutrient release dynamics such as $\text{NH}_4^+\text{-N}$ and $\text{NO}_3^-\text{-N}$ in relation to their initial concentrations of lignin, acid detergent fiber, cellulose and total polyphenol content and their ratios. The dynamic was followed in an incubation pot experiment, complete randomized design in replication. *E. abyssinica* has an average of 4.05, 9.7 and 2.04% TN, lignin and total polyphenol content respectively. *E. brucei* has also an average of 3.05, 12.63 and 1.05% content of TN, lignin and total polyphenol respectively. The samples of *E. abyssinica* and *E. brucei* were ground and incorporated with Luvisol in pots. To determine the amount of ammonium and nitrate released each treatment and control were sampled and analyzed on weekly bases. The lignin and total polyphenol was significantly positively correlated with the release of $\text{NH}_4^+\text{-N}$, while the $\text{NO}_3^-\text{-N}$ showed significant negative correlations with the release of ammonium. From the experiment, it was observed that the *E. abyssinica* with lower content of lignin and high in TN has released the nutrients faster whereas *E. brucei* with high lignin and low total polyphenol content released slowly. In general, these leguminous trees released $\text{NH}_4^+\text{-N}$ and $\text{NO}_3^-\text{-N}$ easily because of their high total nitrogen content and low lignin, ADF, cellulose and total polyphenol content. They attained their half-life within 2-3 weeks. Therefore, *E. abyssinica* and *E. brucei* bears fast mineralization as a result they can be used for fast-term correction of crop nutrient demand. However, more detailed researches are needed to synchronize and verify laboratory results with field measurements of their effect on crop production and synchronization of soil nutrient availability and crop demand in different agro ecology and soil types.

Key words: Incubation, lignin, luvisol, total polyphenol, nitrate, ammonium.

INTRODUCTION

Incorporation of agroforestry legumes trees to the soil have been identified as important management practice

to increase soil fertility. Moreover, the influences of litter quality in determining decomposition rate have been

E-mail: abebeabayt@yahoo.com. Tel: +25191146 7175.

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recognized in the tropics since agricultural practice commenced (Yamoah et al., 1986; Swarup, 1987). Plant litter mineralization is biological decomposition of litter by microorganisms and transfer of organic and mineral compounds to the soil (Loranger et al., 2002). Studies on Ethiopian soil and forest revealed that the very wide range of climate, topography, parent material and microorganisms' and the local conditions of the specific areas have assisted in development of different soil types as well as over 6,600 higher plant species including indigenous leguminous and non-leguminous trees (Mesfin, 1998; MoA, 2000; Mohammed, 2003; Bekele, 2007). According to MoA (2000), Luvisol is one of the soil types estimated to cover 64,063.5 km² or 5.8% of the country. In the future agricultural system, application of organic farming systems is important in order to lessen impacts of climate change, decrease soil erosion (Reganold et al., 1987), improve biodiversity (Hole et al., 2005), and enhance soil fertility (Watson et al., 2002).

Organic farming incorporates researched outputs with ecology and cultural agricultural practices based on naturally occurring microbial processes (Shi, 2013). Among the potentially valuable plant species used for organic farming, *Erythrina abyssinica* and *Erythrina brucei*, are leguminous trees with immense use. *E. abyssinica* fixes nitrogen in the roots infected by Rhizobia nodulate (Legesse, 2002) whereas *E. brucei* fixes nitrogen in the leaves with leaf symbiotic N-fixing characteristics (Legesse, 2002; Orwa et al., 2009). The application of these organic nutrient resources can also be directly incorporation in to the soil and allow it to decompose and release nutrients. However, litter decomposition rates are also governed by the initial biochemical composition as lignin, carbon, total nitrogen, cellulose, polyphenol and phosphorus, potassium concentrations and ratios of carbon/nitrogen (C/N), lignin/N, lignocellulose /N, lignin: N.

One of the major decomposition retarding chemical composition of litter is lignin. It represents nearly 30% of the carbon sequestered in plant materials annually (Boerjan et al., 2003). Lignin is a complex organic polymer which is used for structural materials in the support tissues of vascular plants and some algae as well as it is important in the formation of cell walls (Martone et al., 2009; Lebo et al., 2001). The second mineralization retarding chemical constituents are phenolic compounds. These compounds constituting up to 60% of plant dry mass (Cates and Rhoades, 1997). These consist of more than one aromatic ring, bearing one or more hydroxyl functional groups. Once integrated into the soil, it controls below-ground processes, including soil organic matter decomposition and nutrient cycling (Rovira and Vallejo, 2002; Toberman et al., 2010). As outlined by Zhang et al. (2008) the litter chemicals compositions and their ratio are strong predictors of litter decay, which accounts for over 73% of the variation in litter decomposition rates. At large, the reset parameters that can affect the litter

degradation depends on environmental factors, season and soil types and properties, such as soil pH, temperature, soil moisture, oxygen content, bulk density and particle size.

In order to manage and predict the nutrients released from organic residues for crop uptake there is need to understand the N mineralization dynamics of the litter in relation to their major biochemical composition under specific soil type. Leaf litter of different plant species has diverse nutrient release patterns, which are related to quality, season, and environmental factors (Arunachalam et al., 2003; Abiven et al., 2005).

In order to comprehend the effects of litter quality on N release and how the mineralization rate is affected by biochemical composition of *E. abyssinica* and *E. brucei*, in this study litter decomposition were studied though an incubation experiment. The experiment was designed to determine the amount of NH₄⁺ and NO₃⁻ release in Luvisol and correlate with biochemical constituents of both litter types. Moreover, the study investigates the influence of difference in geographical location on the biochemical composition (N, lignin and polyphenol) of the present leguminous tree species.

MATERIALS AND METHODS

Description of the Sampling Sites

Sidama and Wolaita zones are found in southern Ethiopian. Sidama covers 6972.1 km² and lies between 6°14' to 7° 18' N and 37° 92' to 39°19' E. It has an elevation ranging from 502 to 3000 m.a.s.l. The annual mean temperature of the zone ranges between 10.5 to 27.1°C and the annual mean rainfall ranges from 801 to 1600 mm (HMD, 2015). Wolaita covers an area of 4471.3 km². The zone lies on an elevation ranging from 1200 to 2950 m.a.s.l. with annual average temperature of 15.0 to 28.0°C. The area has a bimodal rainfall pattern, with an average annual rainfall of 1300 to 2000 mm distributed over 8 to 9 months (HMD, 2015).

Leaf and soil sampling

Green leaves samples of *E. abyssinica* and *E. brucei* were collected from ten different randomly selected but geo-referenced locations of Sidama and Wolaita zones of southern Ethiopia biased on soil colour (Tables 1 and 2). A composited Dystric Luvisol sample 0-20 cm in depth was collected from Sidama.

Selected soil physical analysis

Hydrometer method was used for the soil particle size analysis (Bouyoucos, 1951). The bulk density of the sample was determined from sample collected using core ring sampler. The sample was dried at 105° for 24 h, then weighed and calculated accordingly. The plant available soil water holding capacity was determined after determining the field capacity (FC) and permanent wilting point (PWP) of the Luvisol as described by Hillel 1980).

Selected soil chemical analyses

Soil pH and electrical conductivity were measured using an extract

Table 1. *E. abyssinica* sampling sites.

Site	N	E	masl
Aleta Wondo	06° 35'48.2''	38° 25'36.6''	1941
Aleta Wondo	06° 34'53.2''	38° 26'39.3''	2234
Aleta Wondo	06° 34' 56.7''	38° 23'53.7 ''	2483
Titecha	06° 33' 28.2''	38° 31'28.5 ''	2686
Hula	06° 29'26.1''	038° 30'45.3''	2767

Table 2. *E. brucei* sampling sites.

Site	N	E	m.a.s.l
Delbo Atwero	06° 54' 34.2''	37°49' 04.0''	2236
Doga	06° 58' 26.0''	37°52' 25.7''	1975
Gacheno	07° 02' 37.7''	37°55' 33.0''	1884
Kokote	06° 52' 37.7''	37° 35' 33.0''	2154
Shone	07° 09' 34.3''	37° 57' 25.5''	1996

**Figure 1.** Partial view of the incubation experiment set up in greenhouse.

1:2.5 (soil: water) as described by Reeuwijk, (2002). Soil organic carbon content and available P were determined using Walkley and Black (1934) chromic acid wet oxidation method and Olsen and Sommer (1982) based on alkaline extraction by 0.5N NaHCO₃ methods respectively. The total N content in the soil was determined according to Reeuwijk, (2002). Mineral N content (NH₄⁺ and NO₃⁻) was extracted at a ratio of 1:4 (soil: 2M KCl) and determined according to Keeney and Nelson (1982).

Determination of lignin and cellulose via acid detergent fiber (ADF)

Klasson method referenced in Browning (1967) was selected for the analysis of lignin and cellulose via ADF. ADF is prepared from organic nutrient sources material by boiling with sulphuric acid solution of cetyltrimethyl ammonium bromide (CTAB) under controlled condition. The CTAB dissolves nearly all nitrogenous constituents, and the acid hydrolyses the starch to residue containing lignin, cellulose and ash as outlined in Clancy and

Wilson (1966). The 72% H₂SO₄ destroys cellulose; lignin is then determined upon ashing by weight-loss.

Incubation of soil samples

From the composite soil sample two hundred gram for each pot was weighed and mixed with 0.127 g and 0.169 g of *E. abyssinica* and *E. brucei* respectively followed by fully homogenizing in complete random design in replication (Figure 1). Then watering to field capacity was made every day or two until the end of the experiment. On weekly bases, analyses were done to determine, the amount of ammonium and nitrate released from each treatment and control. The analysis were conducted in soil chemistry laboratory using the standard methods.

Statistical

The data obtained from the laboratory analyses of litters and

Table 3. Selected soil chemical and physical characteristics of Luvisol.

Depth cm	pH-H ₂ O 1:2.5	Av.P mg kg ⁻¹	TN %	OC	C/N Mg m ⁻³	BD	FC V/V %	PWP	Sand	Clay %	Silt	Textural class
0-20	4.98	5.32	0.16	1.76	11	1.23	46.20	31.55	14	32	54	Clay

Table 4. Major chemical constituents of *E. abyssinica* and *E. brucei*.

Plant type	TN (%)	K (%)	P (%)
<i>E. abyssinica</i>	4.05 ^a (3.16-5.16)	2.02 ^b (1.94-2.08)	0.39 ^a (0.36-0.43)
<i>E. brucei</i>	3.36 ^b (2.70-3.93)	2.61 ^a (2.54-2.68)	0.31 ^b (0.30-0.32)
LSD (0.05)	0.70	0.36	0.05
CV (%)	13.01	6.32	8.01

*Note: Means in a column followed by the same superscript letters are not significantly different.

**Values in brackets shows range.

Table 5. *E. abyssinica*'s sampling sites and their total nitrogen content.

Site	Altitude (m.a.s.l)	<i>E. abyssinica</i> TN (%)
Aleta Wondo	1941	5.13 ^a (5.16-5.08)
Aleta Wondo	2234	3.26 ^d (3.16-3.31)
Aleta Wondo	2483	4.21 ^b (3.85-4.31)
Titecha	2686	3.67 ^c (3.62-3.70)
Hula	2767	3.98 ^b (3.85-4.08)
LSD (0.05)		0.29
CV (%)		3.97

*Note: Means in a column followed by the same superscript letters are not significantly different.

**Values in brackets shows range

mineralization were subjected to analysis of variance (ANOVA) using statistical analysis software version 9.3 (SAS Institute, 2003). The least significant difference (LSD) was worked to separate means at $p \leq 0.05$ using Duncan Multiple Range Test. To measure release of nutrients (Ammonium and Nitrate) in soil, simple correlation analysis (at $p \leq 0.05$) was carried out.

RESULTS AND DISCUSSION

Phsico-chemical property of Soil used for mineralization

The soil particle size analysis of experiment soil sample was found to be clayey. The critical bulk density value for agricultural use according to Hillel (1980) is 1.4 g cm^{-3} , implying that there is no excessive compaction and restriction to root development. The bulk density of the Luvisol was 1.23 g cm^{-3} . As described by Werner (1997) the soils in this range possesses good porosity for aerobic microorganisms' activities. According to Landon (1996) rating the OC and TN contents of the soil fall in

the "very low" and "low" categorized respectively. The soil is also rated as "low" in available P according to Havlin et al. (2010) rating. Since the soil is at low pH, it possesses phosphorus fixation as well (Table 3).

Litter quality rating of *E. abyssinica* and *E. brucei* with respect to altitude

In this study the analyses of variance for *E. abyssinica* and *E. brucei* showed significant variation in TN content with altitude and sampling site. Sangiga and Woomer (2009) indicated organic nutrients sources with higher than 2.5% TN content are considered high quality. Thus, the average TN content of *E. abyssinica* (4.05%) and *E. brucei* (3.36%) are categorized with the highest N₂ fixing plants (Table 4). The highest mean value in TN content for *E. abyssinica* was obtained at 1941 m.a.s.l (5.16%) and the lowest mean value (3.26%) was found at 2234 m.a.s.l (Table 5). Whereas the highest mean value of TN content (3.93%) of *E. brucei* was obtained at 2154 m.a.s.l

Table 6. *E. brucei*'s sampling sites and their total nitrogen content.

Site	Altitude (m.a.s.l)	<i>E. brucei</i> TN(%)
Delbo Atwero	2236	3.85 ^a (3.77-3.93)
Doga	1975	3.38 ^b (3.31-3.47)
Gacheno	1884	2.85 ^c (2.70-3.00)
Kokate	2154	3.93 ^a (3.91-3.92)
Shone	1996	2.77 ^c (2.70-2.85)
LSD (0.05)		0.17
CV (%)		2.71

*Note: Means in a column followed by the same superscript letters are not significantly different.

**Values in brackets shows range

Table 7. Chemical characterization of *E. abyssinica* and *E. brucei*.

Litter	TPP	ADF	Lignin	Cellulose	TN	C:N	ADF : N	L:N	TPP:N
				(%)					
<i>E. abyssinica</i>	2.04 ^a	30.57 ^b	9.70 ^b	1.10 ^b	4.15 ^a	10.44	12.07	2.34	0.49
<i>E. brucei</i>	1.05 ^b	34.37 ^a	12.63 ^a	1.80 ^a	3.35 ^b	12.26	10.25	3.77	1.24
CV (%)	0.83	1.83	1.77	12.53	1.50				
LSD(0.05)	0.017	1.17	0.34	0.29	0.10				

Where: L–Lignin, TPP- Total Polyphenol, Cellulose– C, TN- Total Nitrogen, ADF- Acid Detergent Fiber.

and the lowest mean value (2.77%) at 1996 (Table 6). As shown on Tables 5 and 6 the variation in TN content of *E. brucei* and *E. abyssinica* can be due to cumulative contribution of micro agroclimatic factors: soil fertility, temperature, and microorganism, in addition to the differences in altitude. The existence of different soil orders might have contributed to the difference in TN content.

E. abyssinica is much better nitrogen fixers as compared to the common organic nutrient sources as referred by Mugendi et al. (2011) which are *Leuceana diversifolia* 3.9%, *Crotalaria juncea* 3.9%, *Vicia benghalensis* 3.7%, *Mucuna pruriens* 4.0%, *Calliandra calothyrsus* 3.4%, *Crotalaria ochroleuca* 4.5%.

E. abyssinica and *E. brucei* had diverse TN content and the data obtained in this study showed that no direct or inverse relationship was found between total nitrogen content and altitude. There was significant difference in TP content between EB and EA. The results revealed that the percentage TP content in EA was higher and significantly different from EB. This difference may be due to the P extracting ability of EA. Both of the organic sources were significantly different with respect to K content ($P < 0.05$) too (Table 4).

Total polyphenol content

The highest total polyphenol (TPP) content was found in

E. abyssinica (2.04%) and the lowest in *E. brucei* (1.06%) (Table 7). The study conducted by Palm and Sanchez (1990) and Anis et al. (2012) also indicated that the primary factors that determines the rate of mineralization and activity of decomposers are the litter quality parameters, especially N, lignin and polyphenol content by inhibiting function of the decomposer organisms by chemically binding to proteins. The study conducted by Grubešić et al. (2005) and Srisuda et al. (2008) indicated that the TPP content of pigeon pea leaves and leaves of *Pouteria altissima* were 2.31%, 4.55 % respectively. The TPP contents in different parts of *Plantago holostium* were: leaves 10.15%, stems 4.13 %, and flowers 3.91% which are higher than the present study spp that is, *E. abyssinica* and *E. brucei*. Therefore, based on their low TPP content it can be said that these plants can undergo in fast decomposition rate.

Total lignin content

E. brucei litters contained the highest (12.63%) lignin content whereas *E. abyssinica* had the lowest (9.70%) of both. Leaf quality have generally been described as high-quality materials in terms of high N and low-lignin contents (Sakala et al., 2000), high ADF and (lignin + polyphenol): N ratio. In general, increasing lignin concentration reduces the residue decomposition rate as outlined by Tian et al. (1992). However, many researches

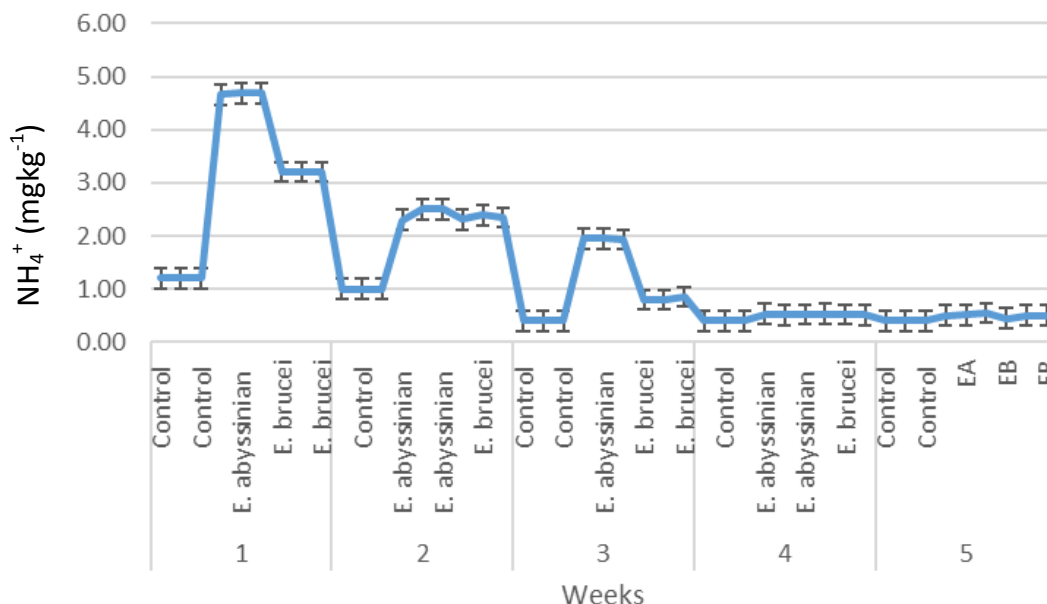


Figure 2. Weekly NH₄⁺-N mineralization of *E. abyssinica* and *E. brucei* in Luvisol.

do not agree on Tian generalization's. They had tried to show by conducting different researched evidences. Among these scholars Stump and Binkley (1993) referred to the lower lignin/N ratio as rate mineralization determining step rather than lignin or nitrogen alone. Thus, according to Tian et al. (1992) study the decomposition order of the litters under investigation shall be *E. brucei* first followed by *E. abyssinica*. However according to Stump and Binkley (1993) the order of mineralization will be *E. abyssinica* first followed by *E. brucei*.

Furthermore, others researchers concluded that it was not the only ratio (lignin/N) that regulates mineralization but other ratios also regulate mineralization (such ratios are shown in Table 7). Handayanto et al. (1997) noted that the initial lignin+polyphenol:N ratios was a good predictor strongly for N mineralization rates or N accumulation when dealing with complex materials. Following the conclusions of these authors, the order of mineralization for the spp under investigations shall be *E. abyssinica* followed by *E. brucei*. However, other researchers such as Probert et al. (2004) and Teklay et al. (2007) concluded that initial N concentration or C:N ratio of residues was the main factor controlling decomposition and nutrient release. These ratios are commonly used to determine the quality of the ONS, that is, the higher the ratio the lower the quality. Moreover, other chemical constituents like potassium (Zaharah and Bah, 1999), phosphorus (Goya et al., 2008) of the litter has a great role on the decomposition rate. From this study, we can infer that the high TN content, low TPP and lignin content of *E. abyssinica* and *E. brucei* as compared to other spp helps to undergo through fast mineralization.

Mineral N release from *E. abyssinica* and *E. brucei* on luvisol

NH₄⁺-N release

NH₄⁺-N is the initial by-product of organic N mineralization. Through the incubation experiment, the highest release NH₄⁺-N (4.68 mg kg⁻¹) and the lowest release (0.48 mg kg⁻¹) was recorded from *E. abyssinica* and *E. brucei*, respectively. However, the laboratory results revealed that there was general decreasing trend in the release of NH₄⁺-N. During the decomposition process each of the litters had showed significant difference with respect to NH₄⁺-N content and the change in NH₄⁺-N was significantly influenced by time of incorporation ($r = -0.90784$; $p < 0.0001$). The NH₄⁺-N released in Luvisol differed significantly at each sampling period (Figures 2 and Table 8). The levels of accumulated NH₄⁺-N released from *E. abyssinica* and *E. brucei* were significantly similar ($r = 0.9374$, $p < 0.5150$) at each week of incubation. The probable reasons for the difference in mineralization of these spp could be the difference in residue quality, total nitrogen content and microbial activity. The results are also in line with that of Schomberg et al. (2009) who stated that the decomposition rate of residues is often influenced by temperature, soil moisture, legume quality parameters such as N, polyphenol, and lignin contents and their ratios regulated by environmental factors. As outlined in Giller and Cadisch (1997) physical accessibility for microbes may also be an important determinant of decay rate, as was observed by increase in microbial activity or decomposition once litter is ground.

Table 8. Interaction effect of Luvisol, *E. abyssinica*, and *E. brucei* and weeks on NH_4^+ -N release (mg kg^{-1}).

Week	Control.	<i>E. abyssinica</i>	<i>E. brucei</i>
1	1.200 ^e	4.680 ^a	3.200 ^b
2	1.000 ^f	2.433 ^c	2.350 ^c
3	0.400 ⁱ	1.943 ^d	0.813 ^g
4	0.400 ⁱ	0.520 ^h	0.520 ^h
5	0.400 ⁱ	0.520 ^h	0.483 ^h
LSD	(0.05)	0.033	
CV (%)	2.49		

Table 9. Interaction effect of Luvisol, *E. abyssinica*, *E. brucei* and weeks incubation on NO_3^- -N release (mg kg^{-1}).

Week	Control	<i>E. abyssinica</i>	<i>E. brucei</i>
1	1.100 ^f	1.917 ^d	1.333 ^e
2	0.950 ^g	2.560 ^c	2.447 ^c
3	0.943 ^g	3.050 ^a	2.533 ^c
4	0.950 ^g	3.080 ^a	2.550 ^c
5	0.950 ^g	2.750 ^b	2.467 ^c
LSD (0.05)		0.0284	
CV (%)		1.928	

Note: Means in a column followed by the same superscript letters are not significantly different at $p < 0.05$.

NO_3^- -N release

During the five weeks' greenhouse mineralization experiment on Luvisol, the experiment revealed that the initial NH_4^+ -N and NO_3^- -N contents were affected by mineralization and nitrification process. The decomposition of *E. abyssinica* and *E. brucei* had similarities in terms of NO_3^- -N release pattern and the change in NO_3^- -N was influenced by both litter types ($r = 0.9243$; $p < 0.0238$), and weeks of incorporation (Table 9).

During incubation period, the nitrification of the control was at the lower rate, compared to the amended soil, where the average release of NO_3^- was in the order of *E. abyssinica* > *E. brucei* (Figure 3). Positive correlation ($r = 0.6571$, $p < 0.0283$) was found between period of incubation (weeks) and NO_3^- -N release in the soil, indicating that there was good association of weeks (incubation) and release of NO_3^- -N. Nitrification was significantly and negatively correlated with ammonification ($r = -0.6571$, $p \leq 0.784$). The decline in the amount of NO_3^- -N starting the end of the experiment and latter might be caused by leaching (because of its high solubility) and denitrification. The soil's cation exchange site does not retain the negative charge, NO_3^- -N, but easily lost from the root zone by leaching and inherent microbes potentially, which accelerates the process of

denitrification (Rochette et al., 2000). Khalil et al. (2002) had observed the application of organic residues produced mineral N in the form of NO_3^- under neutral and slightly alkaline conditions. This study also showed an increase in NO_3^- was dependent on the nitrogen content of the litters. Thus, the increase in NO_3^- -N content was in the order of *E. abyssinica* > *E. brucei* until denitrification commence (Figure 4).

Conclusion

Present results clearly indicate that these species are categorized as the fast decomposing organic nutrient resources with highest TN content regardless of sampling site. *E. abyssinica* and *E. brucei* had diverse TN content and the data obtained in this study showed that no direct or inverse relationship was found between TN content and altitude but other factors could govern the differences in TN content of the organic sources. The faster decomposition and NH_4^+ release performance of *E. abyssinica* in the Luvisol can be accounted for its highest TN content and low-ADF concentrations. The better biochemical quality characteristics of *E. abyssinica* had enhanced the decomposition rate. Incorporating *E. abyssinica* and *E. brucei* to Luvisols showed an increase

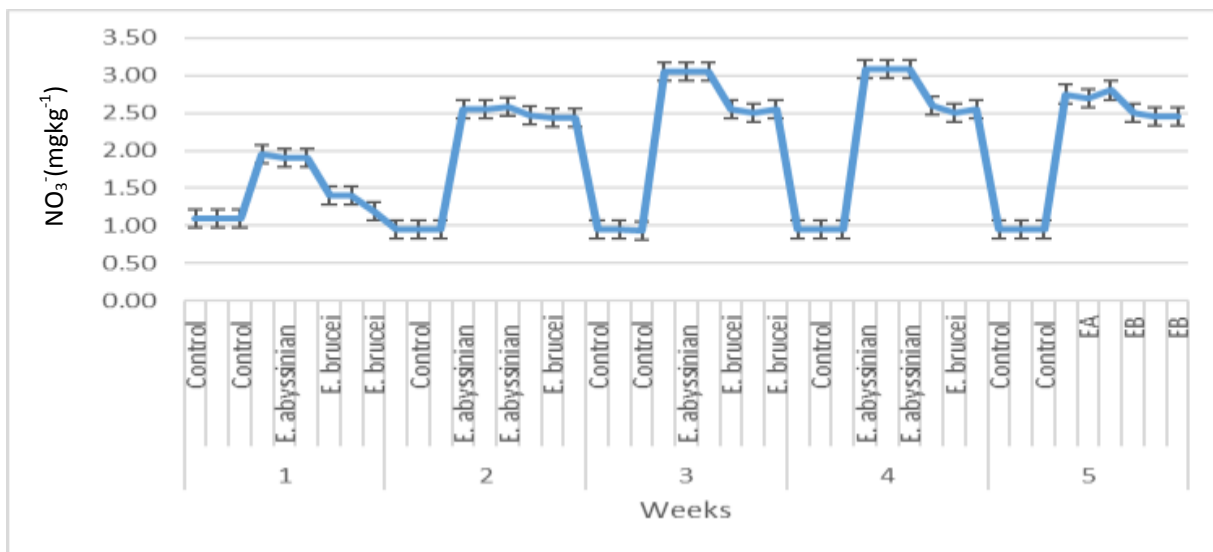


Figure 3. Weekly NO₃⁻-N mineralization from *E. abyssinica* and *E. brucei* in Luvisol.

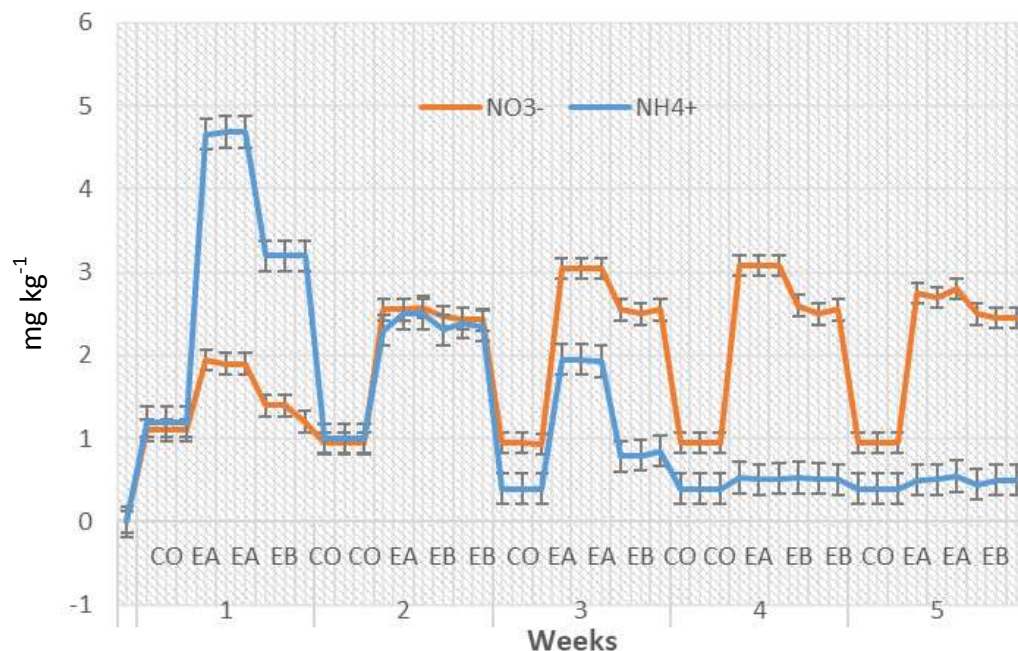


Figure 4. Release of NH₄⁺-N and NO₃⁻-N contents in Luvisol during decomposition. Error bars represent 1 standard deviation. Main and interaction effects were significant at p < 0.05.

in NH₄⁺-N and NO₃⁻-N content of the soil as compared to their respective controls in few weeks. Based on the dynamics of release of NH₄⁺-N and NO₃⁻-N content, the species showed the order: *E. abyssinica* > *E. brucei*.

In general, *E. abyssinica* and *E. brucei* amended soil revealed that NO₃⁻-N release was negatively and significantly correlated with ammonification. During incubation period, the control was at the lower level

compared to the amended soil in ammonium and nitrate concentration.

RECOMMENDATION

Researches that are more detailed are needed to synchronize and verify laboratory results with field

measurements. Their effect on crop production alongside synchronization of different soil properties with crop demand is also necessary.

CONFLICT OF INTERESTS

The author has not declared any conflict of interests.

ACKNOWLEDGMENTS

The author is grateful to their beloved family members for their encouragements and financial support for the study. The support and assistance of Wassie Haile (Ph.D.) and Dhyan Singh (Ph.D.) also deserve appreciations. Finally yet importantly, am also grateful to the anonymous reviewers of the Environment and Natural Resource Research for their constructive comments and suggestions.

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Full Length Research Paper

Effects of waterlogging on growth, biomass and antioxidant enzymes on upper ground and roots of two peony cultivars

Xiangtao Zhu¹, Wen Ji¹, Erman Hong¹, Yufei Cheng¹, Xin Lin¹, Haojie Shi², Xueqin Li¹ and Song Heng Jin^{1*}

¹Jiyang College, Zhejiang A&F University, Zhuji 311800, Zhejiang, China.

²School of Agriculture and Food Science, Zhejiang A&F University, Linan, Hangzhou 311300, Zhejiang, China.

Received 29 September, 2018; Accepted 12 November, 2018

Tree peony (*Paeonia suffruticosa* Andr.) is a perennial deciduous shrub with ornamental and medicinal value. Waterlogging stress is an agricultural problem for peony in Jiangnan of China. This study investigated the growth, biomass, cell membrane permeability and antioxidant enzymes of two *P. suffruticosa* cultivars 'Feng Dan Bai (FDB)' and 'MingXing (MX)'. The response of roots and upper ground to different levels of waterlogging stress in two peony cultivars was also evaluated. Results showed that mild waterlogging stress (MWS) and severe waterlogging stress (SWS) significantly decreased the increment of seedling height, diameter and biomass of leaves. The root biomass increased in FDB but no significant changes in MX. Moreover, cell membrane permeability of leaves and roots also increased, while the chlorophyll content of leaves decreased. Antioxidant enzyme activities of superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT) of leaves and roots all increased, along with a gradual increase in malondialdehyde (MDA). Of this two cultivars, the root system of FDB is more susceptible to waterlogging than the upper ground, and the root system can improve the resistance to waterlogging by increasing the root system biomass, Peony adapted to the waterlogging environment by changing its external form.

Key words: Peony, waterlogging stress, growth, antioxidant systems, malondialdehyde (MDA) content.

INTRODUCTION

Tree peony (*Paeonia suffruticosa* Andr.) is a perennial deciduous shrub of excellent ornamental and medicinal value. It is indigenous to China where ornamental cultivation has a history of more than 2000 years (Li et

al., 2009; Picerno et al., 2011). Because of its large flowers, range of colors, attractive shape and fragrance, tree peony has attracted increasing attention around the world both as a pot plant and for cut flower production

*Corresponding author. E-mail: shjin@zafu.edu.cn.

Xiangtao Zhu, Wen Ji, Erman Hong, Yufei Cheng and Xin Lin contributed equally

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(Han et al., 2008). There are nearly 3000 cultivars of peony in China. Four large tree peony groups have been described of the central, northwest, southwest, and Jiangnan peony groups; the variety of peony in Jiangnan group are the least (Zhang et al., 2007). Peony has large flowers of ornamental value. In recent years, oil peony has also been suggested as an important oil crop. Understanding the factors affecting peony cultivation in Jiangnan of China is important in terms of cultivation management. Changing rainfall patterns have resulted in increased flood events in many regions, so that development of flood tolerant crops is a priority (Yamauchi et al., 2017). Jiangnan of China has a subtropical to tropical monsoon climate. Rainfall is also frequent and heavy during the peony growth period. As a result, the duration of waterlogging is relatively long, often up to 6 months (from April to September), the growth of peony was affected by the waterlogging. Because of this climate, only about 20 peony cultivars remain in Jiangnan group, with some rare cultivars such as 'Zi Yunfang' and 'fengwei' are on the verge of extinction. Therefore, understanding the characteristics of waterlogging tolerance in peony is important in terms of selecting cultivars suitable for growth in Jiangnan regions of China. In line with this, determining the physiological and biochemical characteristics under waterlogging stress is important from both a theoretical and practical viewpoint.

Waterlogging is a major abiotic stress to plants. Globally, it is estimated that 10% of all irrigated land is affected by waterlogging, which might reduce crop productivity as much as 20% (Ren et al., 2016). Waterlogging disturbs plants growth and development, delays growth process, leading to a significant morphological response to stress (Ghobadi et al., 2017; Sauter, 2013; Huang et al., 2015). Waterlogging results in an anaerobic environment, which inhibits aerobic respiration in the mitochondria, inducing anaerobic respiration in the root system. As a result, electron transport is blocked and ATP cannot be produced via the aerobic pathway and the cells rapidly suffer an energy crisis that can lead to cell death, resulting in the accumulation of a large amount of reactive oxygen species (ROS) (Le et al., 2017; Jin et al., 2010; Lesk et al., 2016). Overproduction ROS and subsequent oxidative stress may be the common mechanism of phytotoxicity and cause of damage to important organic constituent of plant cells (Petrov et al., 2015). To eliminate the toxic effects of ROS, plants have different enzymatic or non-enzymatic antioxidants, signaling pathways and metabolites (Ahammed et al., 2013). Rapid biochemical changes are easily induced through short-term soil waterlogging, however, anatomical and morphological changes such as the formation of adventitious roots, hypertrophied lenticels and aerenchyma are more likely to be involved in long-term acclimation (Yamauchi et al., 2017).

Plant responses to waterlogging also vary by species,

genetic characteristics, age, waterlogging duration and waterlogging depth (Zhou et al., 2017; Nyman and Lindau, 2016). While extensive research has been carried out on ROS-induced injury and plant defense response systems under waterlogging stress (Wang et al., 2016; Pocięcha et al., 2016). Roots and upper ground part of plant will be destroyed when plants are under waterlogging stress; little is known about the relationship between roots and upper ground parts. The physiological and biochemical changes in peony under different levels of waterlogging stress were studied (Kong, 2011), but little is known about the differences between water-tolerant species and water-sensitive species. Long-term field observations suggest that the 'Feng Dan Bai (FDB) cultivar is highly tolerant of natural waterlogging conditions. In contrast, the MX is unable to survive for long time under waterlogging stress. Despite of this, little is known about how waterlogging affect growth, morphology, physiological and biochemical characteristics in Jiangnan of China and wetland areas.

The purposes of this research were the following: (1) assessment of the damage of waterlogging in tolerant and sensitive peony used in the region in different degree of waterlogging separately; (2) evaluation of quantitative traits such as roots and upper ground parts growth and other physiological characteristics; and (3) study of stress tolerance indexes in peony varieties, clarifying the mechanisms of waterlogging tolerance in peony. The results of this study will provide a theoretical basis for breeding and cultivation of waterlogging-tolerant cultivars suitable for growth in the Jiangnan of China.

MATERIALS AND METHODS

Plant and growth conditions

Experiments were conducted in Zhejiang A & F University, Zhejiang Province, China (N29°71', E120°23'). Four-year-old healthy FDB and MingXing (MX) seedlings were planted in plastic containers (top diameter: 27 cm; height: 22 cm). A completely randomized design was followed with three replications per treatment and three plants per replication. Containers (18) were used in this experiment. Each container was filled with a mixed matrix consisting of garden soil, sand and perlite (v/v/v = 5: 3: 2, pH 6.4), the depth of soil was 16 cm and grown in a shaded greenhouse with natural sunlight during the day and relative humidity of 65% (±5%). The temperature of greenhouse was 20 to 25°C.

Experimental treatment

In June 2016, three waterlogging stress treatments were implemented as follows: control, standard nutrient-water management with a soil moisture content of 75% field capacity (control by weigh); mild waterlogging stress (MWS), with a flood height of 4 to 5 cm lower than the soil surface; severe waterlogging stress (SWS), with a supersaturated soil water content of 4 to 5 cm above the soil surface. A completely randomized design was followed with three replications per treatment and three plants per replication. Containers (18) were used in this experiment. All other environmental conditions were kept constant throughout the

experiment. All measurements were determined simultaneously after 15-days treatment.

Determination of growth parameters

Seedling height was measured with a tape and ground diameter with a vernier caliper at a height of 6 cm from the soil surface. Each plant was measured twice then the average determined. After measuring height and ground diameter, one intact plant per each treatment replicate was uprooted for roots and upper ground biomass analysis. Green tissues and roots were oven-dried at 65°C to a constant weight then weighed using an electronic scale to determine biomass. All measurements were determined simultaneously after 15-days treatment.

Measurement of leaf chlorophyll

The chlorophyll content was measured using the portable chlorophyll apparatus (SPAD-502Plus).

Cell membrane permeability

Membrane permeability was estimated by measuring the relative electrolyte conductivity in the leaves and roots according to Shi et al., 2006. Discs (0.2 g) were briefly rinsed with deionized water then immersed in a test tube with 30 mL deionized water for 12 h. Initial electrical conductivity (EC) of the solution was subsequently measured with a conductivity meter (Model DJS-1C, Leici, Shanghai). The samples were then heated at 100°C for 20 min and final EC in the bathing solution re-read. Membrane permeability was calculated as $EC (\%) = \text{initial EC} / \text{final EC} \times 100\%$.

Superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT) activities

Leaf tissue samples (0.5 g) and roots (0.5 g) were cut into pieces then ground in 10 ml of 50 mmol phosphate buffer (pH 7.8) containing 1% (w/v) polyvinylpyrrolidone (PVP), respectively. The homogenate was centrifuged at 10,000 × g for 15 min at 4°C, and the supernatant used to determine SOD and POD activities. SOD activity was determined based on the inhibition of nitroblue tetrazolium reduction to blue formazan via superoxide radicals (Ries, 1977). The reaction mixture (3 ml) consisted of 50 mmol potassium phosphate buffer (pH 7.8) with 0.3 mol ethylene diaminetetraacetic acid, 39.15 mol methionine, 0.225 mol nitroblue tetrazolium, 0.006 mol riboflavin and 0.05 ml enzyme extract.

POD activity was determined using the guaiacol method (Sun et al., 2011). The reaction mixture (3 ml) contained 0.05 ml enzyme extract, 2.75 ml of 50 mmol phosphate buffer (pH 7.0), 0.1 ml of 1% H₂O₂ and 0.1 mL of 4% guaiacol solution. The increase in absorbance at 470 nm due to guaiacol oxidation was recorded for 2 min then one unit of enzyme activity defined as the amount of enzyme causing a change in absorbance of 0.01 per min.

CAT activity was determined by tracking the consumption of H₂O₂ at 240 nm for 3 min (Aeby, 1984). The assay mixture (3 ml) consisted of 100 mmol potassium phosphate buffer (pH 7.0), 15 mmol H₂O₂ and 50 ul leaf extract.

Malondialdehyde (MDA) content

MDA content was determined according to the method of Jin et al. (2011). The degree of lipid peroxidation was determined from the content of 2-thiobarbituric acid (TBA) reactive metabolites. Fresh

leaf tissue and roots were respectively homogenized and then extracted in 10 ml of 0.25% (w/v) TBA dissolved in 10% (w/v) trichloroacetic acid (TCA). The extract was heated to 95°C for 30 min then cooled quickly on ice. Absorbance of the supernatant was measured at 532 nm after centrifugation at 10,000 × g for 10 min, and correction of non-specific turbidity carried out by subtracting the absorbance at 600 nm.

Statistical analysis

Data were analyzed using analysis of variance (ANOVA) with SPSS version 19.0. Means were separated by calculating the least significant difference (LSD), and simple correlation analysis to determine the relationship between each physiological variable.

RESULTS

The growth of roots and upper ground of peony during waterlogging stress

The results illustrated the effects of waterlogging on peony growth (Figure 1). The increment of height, ground diameter and upper ground biomass was decreased as the increasing waterlogging stress, but the increment of roots biomass increased in FDB and has no significant difference in MX. Moreover, the increases in height, diameter, upper ground biomass under waterlogging stress were lower than the control of two cultivars, but the roots biomass were higher than control in FDB. Values of each morphological indicator were also lower in MX than FDB, suggesting that FDB was less impacted by waterlogging stress than MX. In two cultivars, the increase in ground diameter was lower under MWS than control, but higher than that under SWS, ranging from 0.16 to 0.21. Significant differences in ground diameter between the two cultivars were also observed under stress, MX showing consistently lower values than FDB. The increases in seedling height were also lower in MX than FDB; however, no significant differences in above ground biomass were observed between the MWS and SWS treatments in two cultivars. Roots biomass of FDB increased during MWS and SWS treatments than control; roots biomass of MX has no significant change between MWS and SWS treatments.

Cell membrane permeability and chlorophyll content

As shown in Figure 2, no significant differences in cell membrane permeability in leaves of the two cultivars can be seen under normal management and MWS condition. A gradual increase with increasing waterlogging stress was observed in two cultivars, with a larger increase in MX than FDB. However, under SWS condition, cell membrane permeability was significantly greater in MX than in FDB. The roots cell membrane permeability was also increased as the increase in degree waterlogging treatments. No significant differences

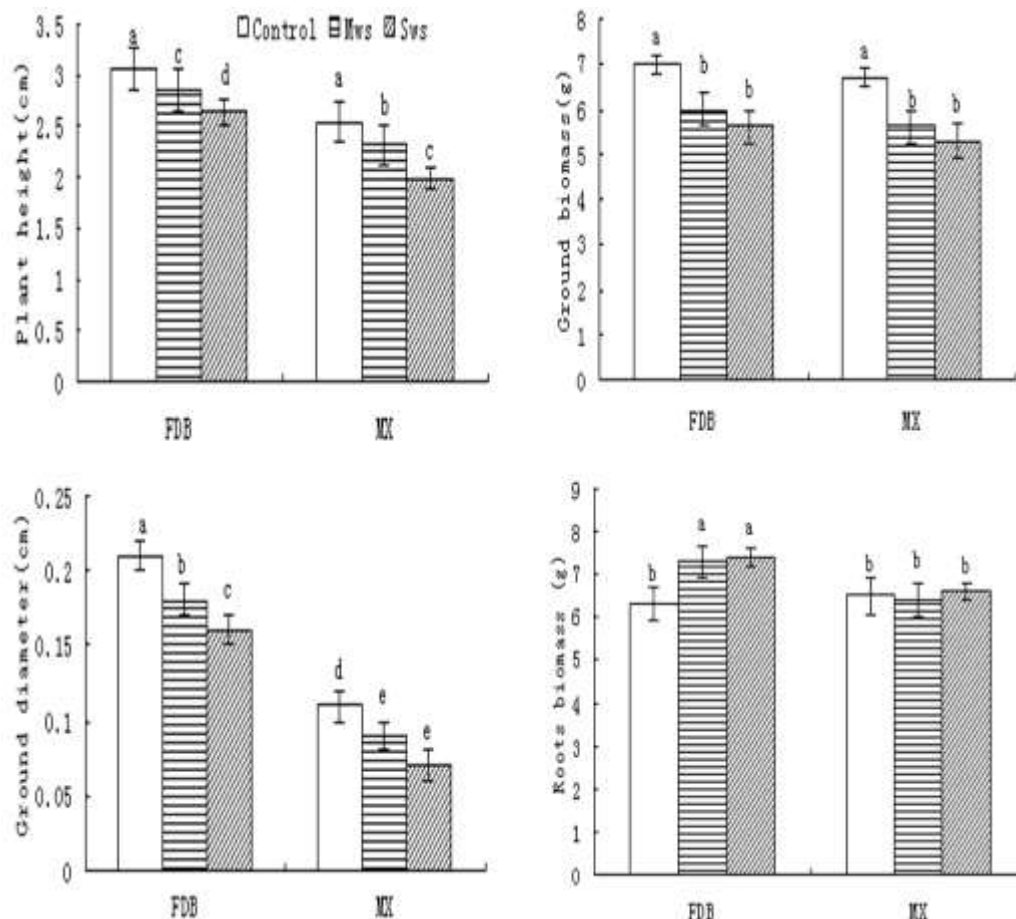


Figure 1. Changes in seedling height, ground diameter, upper ground biomass and roots biomass under waterlogging stress in two peony cultivars. Each bar represents the mean+SE calculated from three independent experiments. Bars with different letters are significantly different at $P < 0.01$.

were observed under control between two cultivars, however, under MWS and SWS treatment, the cell membrane leakage was increased. The increase in cell membrane leakage was significantly lower in FDB than MX (Figure 1). The resistance of peony varieties for waterlogging increases with decrease in percentage of cell membrane leakage.

The chlorophyll content was greater in FDB than MX under all treatments (Figure 2), with a decrease in two cultivars under MWS compared to control, and a further decrease under SWS condition. That is, chlorophyll content decreased in both cultivars with increasing waterlogging stress. No significant differences can be seen between MWS and SWS in FDB; however, significant differences were observed in MX with increasing waterlogging stress.

Antioxidant enzyme activities and MDA content

As shown in Figures 3 and 4, antioxidant enzyme activity of leaves and roots increased gradually with increasing

waterlogging stress, as did the MDA content. Enzyme activities and MDA content were both the highest under severe waterlogging stress, with significant differences as compared to the control ($P < 0.01$).

In this study, SOD activity of leaves increased slightly with increasing waterlogging stress (Figure 3). Under control condition, SOD activity was higher in MX than FDB, under MWS the activity of SOD of two cultivars were higher than the control. Under SWS, an increase of 37.5 and 16.1% compared to the control was observed in FDB and MX, respectively. SOD activity of roots was also increased (Figure 3); under control condition, no significant differences were observed between FDB and MX. Under MWS and SWS condition, SOD activity of roots was increased both in FDB and MX. Above all, when peony is in water, the root system and the upper ground part show the same reflection between two cultivars.

Under waterlogging stress, the changes in CAT activity of roots and leaves were similar between the two cultivars (Figure 3), and the activity of CAT was higher in FDB than MX. In FDB, no significant differences in CAT

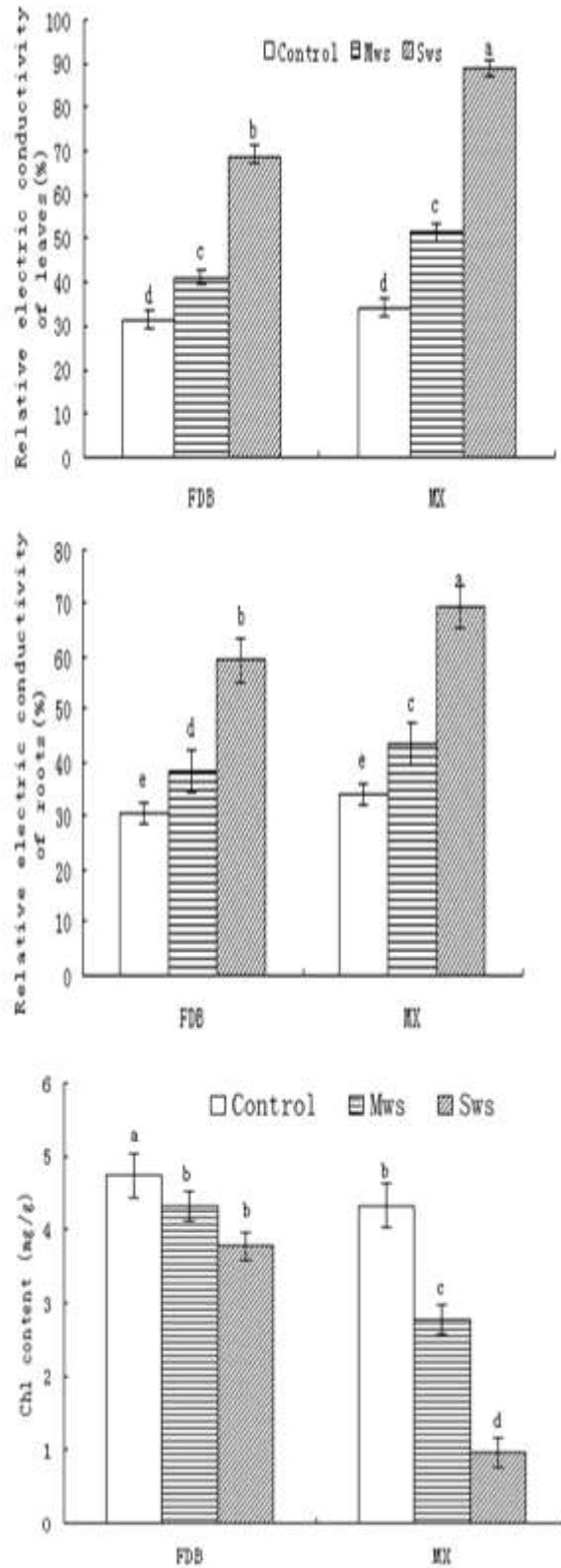


Figure 2. Changes in cell membrane permeability of leaves and roots and chlorophyll content under waterlogging stress in two peony cultivars. Each bar represents the mean+SE calculated from three independent experiments. Bars with different letters are significantly different at P < 0.01.

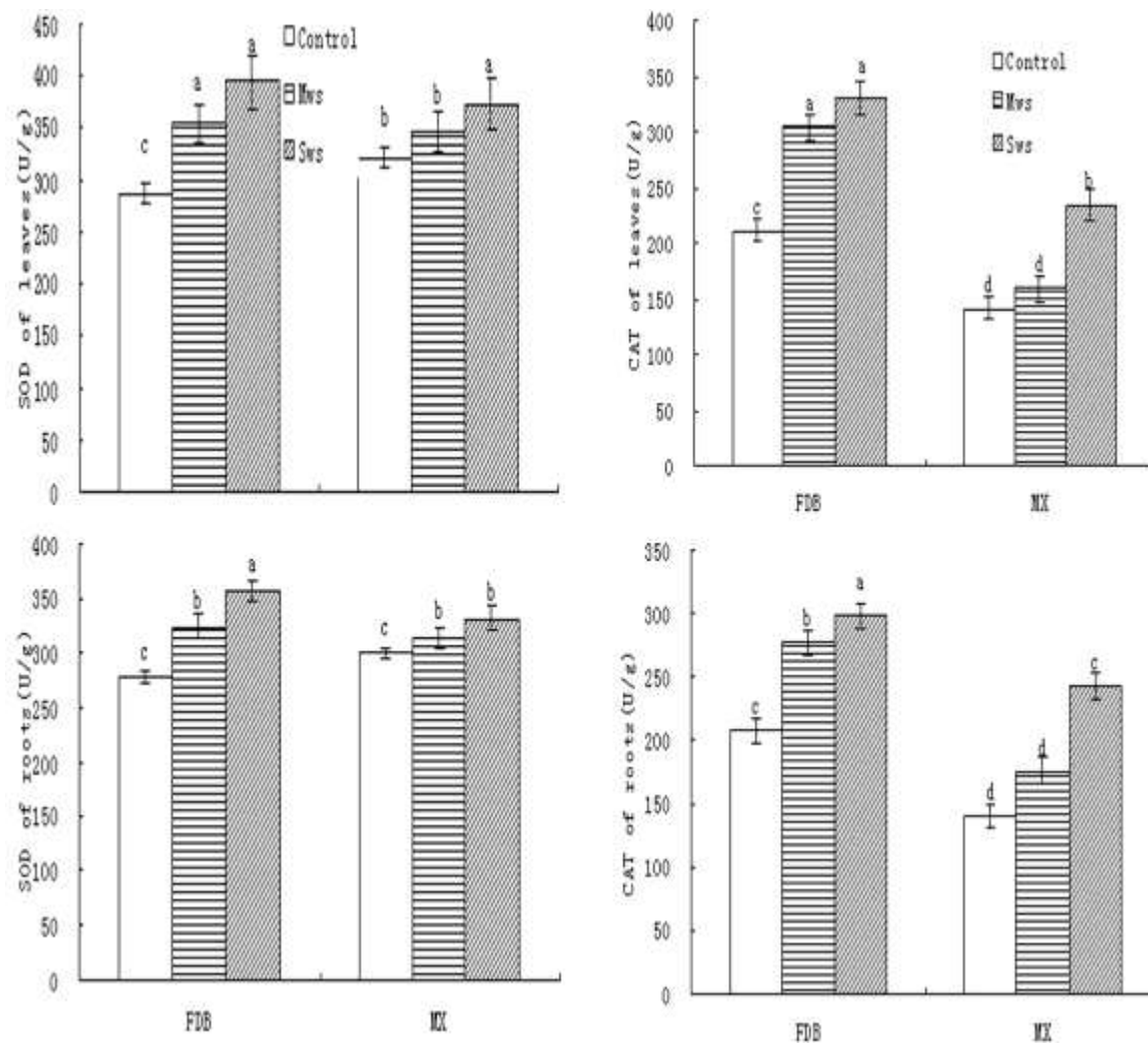


Figure 3. Changes in antioxidant enzyme SOD and CAT activities under waterlogging stress in two peony cultivars. Each bar represents the mean+SE calculated from three independent experiments. Bars with different letters are significantly different at $P < 0.01$.

activity of leaves were observed between MWS and SWS condition; however, significant differences were observed when compared with the control ($P < 0.01$). But significant differences were observed in the CAT activity of roots among control, MWS and SWS ($P < 0.01$). In MX, no significant differences were observed between control and MWS; however, a significant difference was observed under SWS ($P < 0.01$). That is, an increase in CAT activity of 19.9 and 42.1% was observed under SWS compared to control. Under MWS and SWS condition, CAT activities of roots increased by 5% ($P < 0.01$) and 9% ($P < 0.01$) than control, respectively. Above all, it can be observed that when peony is in waterlogging, the root system and the upper ground part show the same reflection.

POD activity of roots and leaves showed a similar trend in two cultivars, increasing trend were observed with increasing waterlogging stress (Figure 4). Under all

treatments, POD activity of leaves were lower in FDB than MX, with significant differences in both cultivars between control, MWS and SWS condition ($P < 0.01$). Under SWS condition, an increase of 58.2 and 35.9% was observed in FDB and MX compared to the control. POD activity of roots was increased with increasing waterlogging stress in FDB and MX, with significant differences observed in two cultivars in control, MWS and SWS conditions ($P < 0.01$). Under SWS condition, an increase of 53.2 and 51.2% was observed in FDB and MX compared to the control, respectively. Similar trend was observed between roots and leaves of two cultivars.

MDA content was found to be increased due to flooding treatment. However, the increase in MDA content of leaves under MWS were significantly lower in FDB (30.7%) followed by MX (34.2%). In SWS condition, an increase of 52.4 and 52.1% was observed in FDB and MX compared to the control. MDA content of roots also

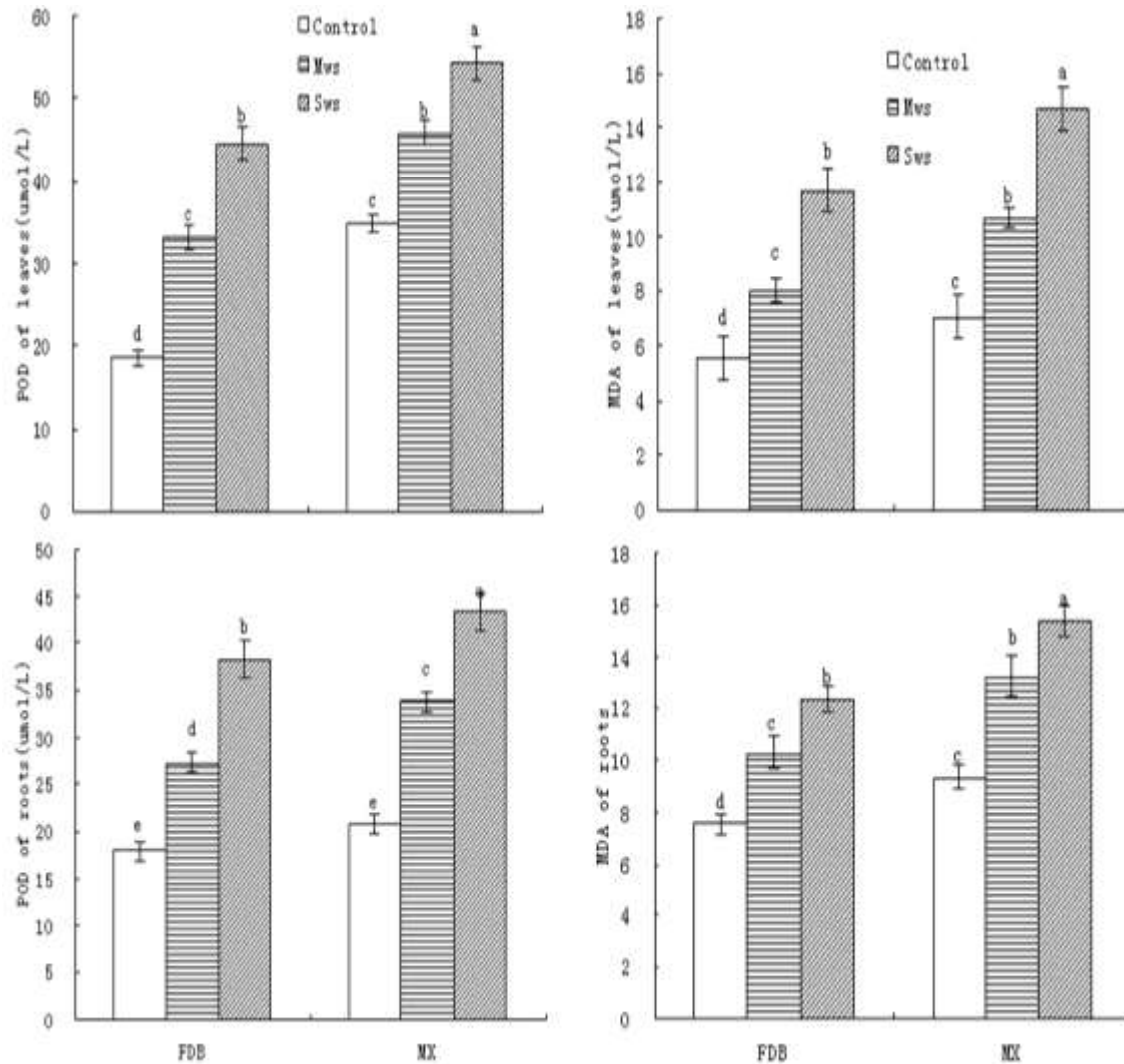


Figure 4. Changes in antioxidant enzyme POD activities and MDA content of leaves and roots under waterlogging stress in two peony cultivars. Each bar represents the mean+SE calculated from three independent experiments. Bars with different letters are significantly different at $P < 0.01$.

increased gradually with increasing waterlogging stress, with significant differences between treatments in two cultivars. Under SWS, an increase of 39.1 and 39.2% was observed in FDB and MX compared to the control.

DISCUSSION

Effects of waterlogging stress on morphological characteristics

Under waterlogging stress, the external morphology of peony undergoes a series of changes, with a decrease in growth rate and gradual increase in biomass, the effect in intolerant cultivars greater than that in tolerant cultivars. In this study, no significant differences in the biomass

increment were observed between FDB and MX with increasing waterlogging stress. In contrast, a greater increase in height was observed in FDB. Overall, FDB was less affected by waterlogging stress, suggesting stronger waterlogging tolerance. The decrease in seedling height, seedling diameter and biomass under waterlogging stress is mainly the result of waterlogging of the soil system, which causes soil hypoxia and a subsequent decrease in root activity (Kong, 2011). Water saturation of the roots results in root anaerobic respiration and subsequent production of harmful substances such as ethanol, thus hindering seedling height and ground diameter growth. Roots are challenged by various abiotic and biotic constraints in soils, with water status of too little or too much being a major factor resulting in plant stress. An increased number of newly-

emerged adventitious roots can compensate, at least partially, for the growth inhibition or even death of distal portions of roots present when waterlogging occurs. Many plant species produce adventitious roots (Visser and Voesenek, 2004), with some emerging into the soil, others along the soil surface and during deeper floods some even grow into the water column. The roots of MX were not increased because the adventitious roots biomass not produced in the experiment. That is to say, MX has no ability to produce new roots when emerged in water. The roots died after a long time water emerged. In the present study, the roots biomass of FDB was increased because of the adventitious roots produced in the water, this is, the reflection of tolerant peony to flooding to alleviate the injury of waterlogging. With the formation of new roots, the respiratory area of the root system gradually increased, which enhanced the waterlogging resistance of peony. The process of the formation of new adventitious roots remains to be further studied in the future.

Effects of waterlogging stress on physiological and biochemical characteristics

Chlorophyll is involved in the absorption, transfer and conversion of light energy during photosynthesis. With an overall decrease in chlorophyll content, light energy conversion and the overall energy supply are inhibited, thereby affecting photosynthesis. Thus, to a large extent, the chlorophyll content of a plant reflects its growth status and photosynthetic capacity. Under stress, the chlorophyll content decreases as a result of changes in cell membrane structure (Cao et al., 2015). Stress also causes an increase in ROS and MDA, thereby accelerating chlorophyll decomposition and further decreasing the overall chlorophyll content (Yi et al., 2008). In this study, chlorophyll content decreased as increasing waterlogging stress, and the MDA content and chlorophyll content showed a negative correlation. These findings confirm the relationship between chlorophyll content and ROS, consistent with a previous study in rice (Jiang et al., 1994). It has been suggested that chlorophyll content under stress may reflect the degree of tolerance (Yi et al., 2008). In this study, the chlorophyll content of FDB was significantly higher than that of MX, suggesting stronger waterlogging tolerance in FDB.

When faced with external environmental stresses, cell membrane permeability increases due to increased leakage of electrolytes (Burgess et al., 2014; Tang et al., 2014). The reason for this was that the cell membrane was damaged under adverse stress and the membrane permeability increased, so that the electrolyte infiltration inside the cell increased the conductivity. Cell membrane damage can therefore be determined by calculating the electrical conductivity of fluid. The higher the electrical conductivity, the greater the leakage of fluid and the more

electrolytes are present, thus, the more serious the cell membrane damage. In this study, electrical conductivity increased with waterlogging stress in two cultivars and was greater in MX than FDB. This results further suggests that MX is more greatly affected, and therefore, less tolerant to waterlogging stress.

Under normal circumstances, there is a dynamic balance between generation and elimination of ROS. However, stress breaks this balance, causing a substantial accumulation in ROS, increasing the generation of MDA (Jin et al., 2010). In turn, this causes further damage to the membrane structure, inducing a series of physiological and biochemical changes (Jin et al., 2010). ROS can be eliminated via antioxidant enzyme activity, alleviating damage to the plant. In antioxidative systems of plants, SOD can remove $O_2^{\cdot-}$. As SOD may control other activated species (H_2O_2 and OH), it is defined as a key antioxidative enzyme in the system. POD is an important enzyme involved in morphogenesis and auxin oxidation. It is the enzyme which is very sensitive to environmental fluctuations being considered as a measure of plant resistance to stress. The main enzymes involved in this process are SOD, POD and CAT (Jin et al., 2011). As shown in this study, SOD, POD and CAT activity increased with increasing waterlogging stress along with MDA content. Thus, under waterlogging stress, peony plants activate an automatic adjustment mechanism, however, at a certain level of stress, the effect on growth and development is unavoidable. SOD initiates membrane lipid peroxidation both directly and indirectly, increasing the content of MDA, the accumulation in MDA in turn inhibits SOD, reducing the protective effects of the enzyme system and further promoting damage.

In this study, the relationship between roots and upper ground were discussed; at the later period of waterlogging, adventitious roots were produced in FDB. Due to the new roots, the tolerant of waterlogging will be strengthened. This study also suggests that this is the main physiological response and damaging effect of soil waterlogging stress in peony. A great difference was observed in waterlogging tolerance between two peony cultivars. Such differences were caused by the different level of chlorophyll and different antioxidant enzyme activities, so that various indices characterizing growth activity, as well as the cell membrane permeability and MDA content, changed to a different degree. The accumulation of MDA in FDB was lower than in MX; it was mainly due to the sharp increase in antioxidant enzymes.

ACKNOWLEDGEMENTS

The work was partly supported by the Zhejiang Provincial Natural Science Foundation of China (LY16C160011), the National Natural Science Foundation of China (Nos.

31170584 and 31200525) and National College students' Innovation and Entrepreneurship Training Program of China (201813283008).

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Full Length Research Paper

Effect of Electroculture on seed germination and growth of *Raphanus sativus* (L)

Mukundraj B. Patil

Department of Botany, Late Ramesh Warpukar College, Sonpeth, Dist. Parbhani (MS) -431 516, India.

Received 17 September, 2018; Accepted 12 November, 2018

Experiments were carried out to study the effect of electricity on the seed germination and growth of the *Raphanus sativus*. For the experiment, twelve pots were grouped into four sets, each containing three pots. In each pot, 20 seeds were sowed. While keeping all the parameters constant 3V, 6V and 9V electricity was supplied for 10 min daily to three groups only, while one set of three pots were not supplied with the electricity and called as control. Number of seedlings that emerged from soil were counted and percentage germination was calculated. The highest percentage of germination (95%) was recorded in pots supplied with 9V electricity, followed by 90% in the pots supplied with 6V electricity. It was 85% in Control and Pots supplied with 3V electricity. Supply of electricity has significant effect of electricity to increase length of root, diameter of root and weight of root and biomass. Supply of 9V electricity was most effective to affect the growth parameters of *R. sativus*.

Key words: Electricity, growth parameters, percentage germination.

INTRODUCTION

The application of electricity for plant growth is known as Electro-culture. Experimental study of electricity on the plant growth was started late back in the 18th century but their results were contradictory. Different researches have proved that Electro-culture enhance germination of seed (Morar et al., 1999; Palov et al., 2003; Palov and Sirakov, 2004; Patwardhan and Gandhare, 2013; Gui et al., 2013) and growth rates (Murry, 1965; Batchman and Reichmanis, 1973; Cestino et al., 2000; Ahmet, 2003; Rotcharoen et al., 2002; Kiatgamjorn et al., 2002). It is also helpful to increase the yield of the crops (Pittman, 1977; Morar et al., 1999).

Recently, it is also mentioned that Electro-culture can protect plants from diseases, insects and frost. It also can

reduce the requirements for fertilizer or pesticides (Morar et al., 1999; Nelson, 2011). Present investigation was carried out to study the effect of 3V, 6V and 9V electricity on the germination as well as on the growth of the *Raphanus sativus*.

MATERIALS AND METHODS

Experiments were carried out during 1 January 2017 to 15 February 2017. For this experiment, twelve pots were taken in the four sets each containing three pots. Three sets were supplied with 3V, 6V and 9V electricity respectively while fourth set was kept as control. Twenty radish seeds were then planted at equal distance (7cm) from each 5cm away from Copper rod. All the factors (Food, Water,

E-mail- mukundrajpatil@gmail.com.

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Table 1. Effect of electricity on germination of *R. sativus* seeds.

DAI	Control	3volt	6volt	9volt
1	0	0	0	0
2	0	0	6.7	28.33
3	30	33.33	50	65
4	65	70	80	83.33
5	81.67	80	88.33	93.33
6	85	85	90	95
Source	df	SS	MSS	F
Treatments	3	1191.57	397.19	11.82
Days	6	35517.26	5919.54	176.13
Error	18	604.96	33.61	
Total	27	37313.79		

DAI: Days After Incubation
Statistical Analysis: ANNOVA.

Table 2. Effect of electricity on growth of *R. sativus*.

Character	Control	3 V	6 V	9 V	Mean	S.D.	CD 5%	CD 1%
Root Length (cm)	17.25	14.9**	19.16 ^{NS}	20.61 ^{NS}	17.98	2.47	3.93	7.22
Thickness of Root (cm)	1.19	1.53*	1.54*	1.85*	1.53	0.23	0.37	0.68
Weight of Root (gm)	9.96	10.44 ^{NS}	10.87 ^{NS}	14.99*	11.56	2	3.19	5.85
Weight of aerial part in gm (Biomass)	4.15	4.8*	5.95*	6.52*	5.36	0.93	1.48	2.71

NS - Non-Significant; * - Significant at 5% CD; ** - Significant at 1% CD.

and Sunlight) were kept constant except electricity. For electric supply, two copper rods were buried in the soil at opposite sides of the pots in such a way that they could not touch each other. The DC 3V, 6V and 9V was supplied daily for 10 min. Germination was observed daily after sowing until the germination value remains same. Observations were recorded and Mean value of percent germination was calculated (Table 1). One pot from each group containing 10 seedlings each were retained for further study. Supply of electricity was continued daily for 10 min. After 45 days, various growth parameters were studied. Root length was measured with the help of Centimeter scale. Thickness of root was measured (in centimeter) using Vernier caliber exactly at the center of root. The weight of root and weight of aerial parts (Biomass) was measured with the help of electric balance. All values were recorded and mean values were tabulated in Table 2.

RESULTS AND DISCUSSION

The pots not receiving electricity and the pots receiving 3V electricity shows emergence of seedling three days after sowing while pots receiving electricity 6V and 9V electricity shows germination on second day of sowing. Thus, it is clear that external application of the electricity to the *R. sativus* seeds induces earlier germination of seeds.

Maximum germination was recorded on six days after

sowing in control and pots supplied with 3V electricity. However, the Experimental pot supplied with 3V electricity and 9V electricity was 90% and 95% respectively. Thus, percentage germination of the *R. sativus* seeds was increased due to the supply of 6V and 9V electricity to the pots. Analysis of Variation shows that there is significant variation in percentage seed germination as well as germination rate. It significantly increased with the increase in days of germination as well as increasing voltage. Different researchers (Labes, 1993; Pozeliene and Lynekine, 2009; Gandhare and Patwardhan, 2014; Rotcharoen, et al., 2002) recorded such type of positive effect of electrical field on seed germination. Such type of increased germination rate and germination percentage due to application of electricity is attributed to the physiological and biochemical changes (Putincev and Platonova, 1997), such as free radical excitement, increase in the activity of protein and enzymes to increase seed vigor (Morar et al., 1999; Bai et al., 2003).

Growth parameters like root length, thickness of root, weight of root as well as biomass of the aerial parts increased considerably due to the application of electricity. In the control experiment, mean length of root was 17.25 cm; while it was 14.9, 19.16 and 20.61 cm respectively in the pots supplied with 3V, 6V and 9V

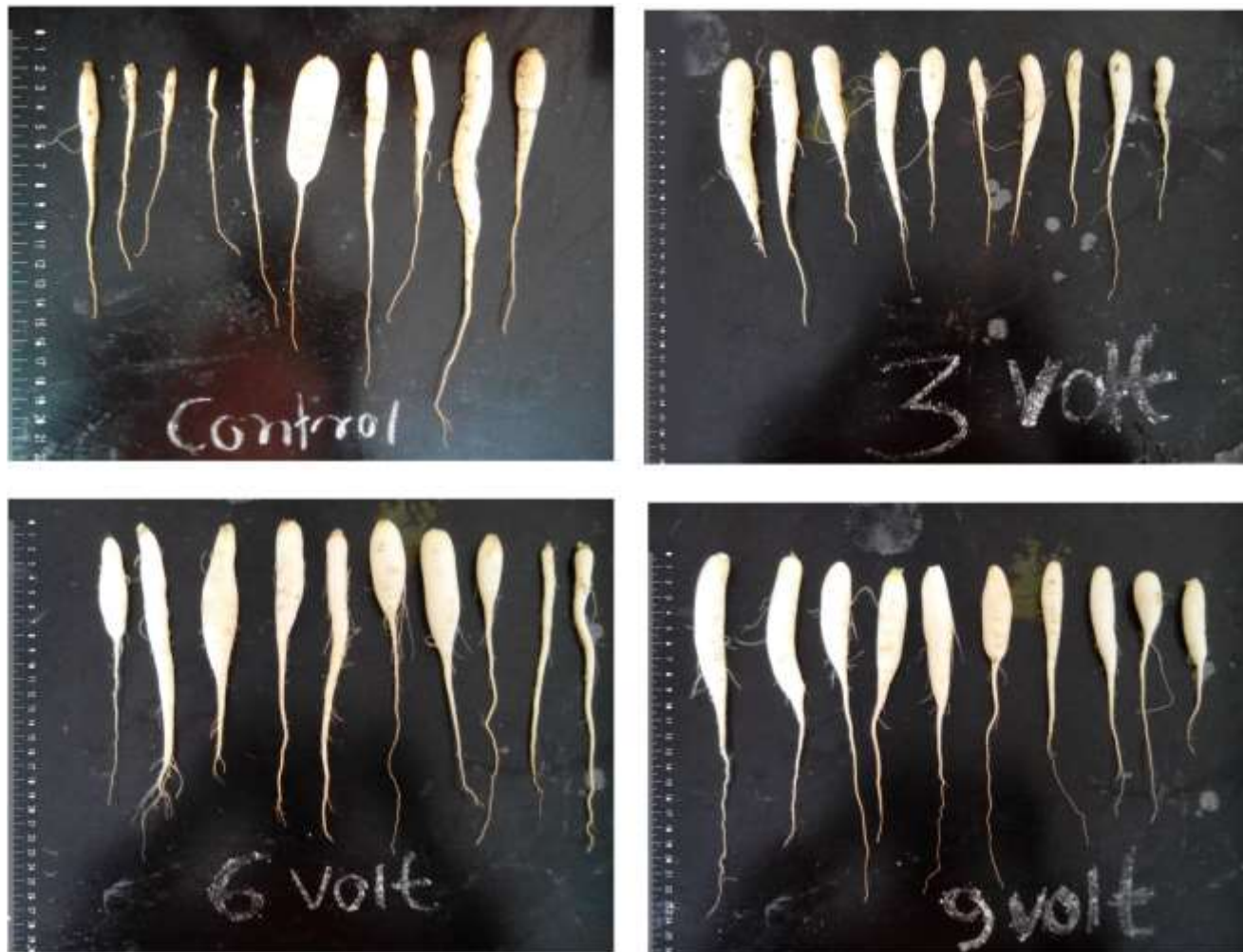


Plate 1. Showing effect of electricity on *R. sativus* root.

electricity respectively (Plate 1). Increase in the root length due to supply of 6V and 9V was non-significant; while decrease in root length in plants supplied with 3 Volts electricity was significant (CD = 1%).

There is increase in the thickness as well as weight of the *Raphanus* root. This increase in the thickness of *Raphanus* root due to application of electricity was statistically significant at CD = 5%. Maximum increase in the weight of root was found in pots supplied with 9V electricity, which was 14.99 g and it was 50.5% greater than the control which was 9.96 g. This increase in the weight was significant at CD = 5% in other cases. Thus, there is an increase in the yield of *R. sativus* due to application of electricity. Ozel (2003) recorded same results in wheat and (Kiatgamjorn et al., 2002) in beans.

This increase in the weight of *Raphanus* (Yield) is because electric field affects the ions in the soil or on the metabolism of electrons and ions (Celestino et al., 2000). This effect of electricity on plant growth may be due to impact of electric field on electron transport chain and the dark and light reactions of photosynthesis (Celestino et

al., 2000; Bachman and Reichmanis, 1973)

Conclusion

The supply of Electricity increases germination rate as well as percentage germination of *Raphanus* seeds. It is also helpful to increase weight of the *Raphanus* root; thus, increasing the yield. Further research should be carried out in this field to provide an alternative for the chemical fertilizers at minimum cost.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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