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Full Length Research Paper

Germination, vigor and pathogen incidence in broccoli seed treated with Carboxin + Thiran

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The purpose of treating seeds chemically is to eradicate their pathogens and/or protect them against soil pathogens, mainly by germination time. However, there is little research on vegetables investigating the effect of this treatment on seed quality. Therefore, this study evaluates the effects of Carboxin + Thiram doses on germination and vigor of three lots of broccoli seeds, as well as on the incidence of fungi in treated seed. The 15 treatments were evaluated in a factorial system (3x5), with the first factor consisting of three lots of 'Avenger' broccoli seeds (lots 82744, 82745 and 82749), and the second factor consisting of five doses (0, 0.04, 0.06, 0.10 and 0.12% of a.i.) of Carboxin + Thiram fungicide (commercial name Vitavax-Thiran). The germination and seed vigor were evaluated, in addition to the presence of pathogens in seeds after treatment (blotter test). All lots showed high levels of germination and vigor. The lot 82749, however, showed higher value in plug test in substrate emergence (99%) than lot 82745 (95%). Regarding the treatment with Carboxin + Thiram, no changes in germination average (98%) and vigor were noticed (average for the first germination count, length, and dry weight of seedling, plug test at 10 days after sowing of 97%, 4.9 cm, 4.0 mg and 96%, respectively), showing that this fungicide, in the evaluated doses, does not affect the quality of broccoli seeds. As to seeds health, the pathogens *Alternaria* spp. and *Fusarium* spp. were detected, in addition to saprophytic species such as *Penicillium*, *Aspergillus*, *Trichoderma,* and *Rhizopus*. The higher incidence of *Fusarium* spp. was noticed in lot 82744, and the lowest in lot 82749. As to *Penicillium* spp., lot 82479 was the most contaminated. Regarding other fungi, the general incidence was very low and there was no difference between lots and doses used.

Key words: *Brassica oleracea*, seed treatment, fungicides.

INTRODUCTION

It is the seed physiological and seed sanitary quality that will determine their performance in the field, that is, the proper establishment of plants, which is essential for satisfactory levels of productivity and final product quality (Nascimento et al., 2011). It is not always possible to obtain seed lots with 100% guaranteed disinfection of

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pathogens. Also, it is not possible to ensure that the soil or substrate will be free of pathogens. Hence, in most cases, treating seeds of vegetables, particularly those whose seeds are of highest value, is required. The purpose of treating seeds chemically is to eradicate their pathogens and/or protect them against soil pathogens, mainly by germination period. Furthermore, as small quantities of products per unit area are used, there is less risk of environmental pollution (Carvalho and Nakagawa, 2000).

According to Menten and Moraes (2010), there were 19 active fungicide ingredients registered for seed treatment in Brazil, although these registration covers some species only. Among those ingredients, one of the most used is the Carboxin + Thiram because, according to Marini et al. (2011), it provides greater protection to seeds against pathogens found in soil and in the seed itself, especially when exposed to unfavorable development conditions.

There are studies assessing the effects of this fungicide on seed physiological quality of cotton (Faria et al., 2003), castor plant (Tropaldi et al., 2010; Santos et al., 2012), peanuts (Bittencourt et al., 2007), rice (Schuch et al., 2006; Lobo, 2008; Morales et al., 2012), maize (Fessel et al., 2003), safflower (Rogério et al., 2012), and wheat (Marini et al., 2011), among other species. However, no research on vegetables was found that studied the fungicide effect on seed quality. The vast majority of vegetable seeds sold in Brazil are treated with fungicides, as their quality increases and the treatment cost is low, mainly for hybrid seeds, whose price is high.

The seed treatment effectiveness depends, among other factors, on the seed species and vigor, which may vary from lot to lot (Menten and Moraes, 2010), and the treatment should not affect the seeds physiological quality. According to Cardoso and Silva (2009), seeds of high physiological quality are essential to brassica production components, as they favor strong, uniform, and healthy seedlings. Therefore, this study evaluates the effects of Carboxin + Thiram doses on germination and vigor of three lots of broccoli seeds, as well as on the incidence of fungi in treated seed.

MATERIALS AND METHODS

The experiment was conducted in the Vegetable Seeds Laboratories of the Horticulture and Plant Protection Departments, of the Universidade Estadual Paulista (UNESP), Botucatu City, São Paulo State, Brazil. The 15 treatments were evaluated from a 3x5 factorial system, with the first factor consisting of three lots of Sakata® 'Avenger' hybrid broccoli seeds (lots 82744, 82745 and 82749), and the second factor consisting of five doses (0, 0.04, 0.06, 0.10 and 0.12% of a.i.) of Carboxin + Thiram.

The commercial product used was VitavaxThiram®, which contain the following active ingredients (a.i.): 5,6-dihydro-2-methyl-1,4-oxathi-ine-3-carboxanilide (Carboxin, 200 g L^{-1}, that is, 20% w/v), and Tetramethylthiuramdisulfide (Thiram 200 g L^{-1}, that is, 20% w/v), and Ethylene Glycol (249 g L^{-1}, that is, 24.9% w/v), and other ingredients (507 g L^{-1}, that is, 50.7% w/v). It is a systemic and contact fungicide of the Carboxanilide (Carboxin) and Dimethyldithiocarbamate (Thiram) chemical group, and used in seed treatment. The evaluated doses correspond to the following commercial product (c.p.) doses: 0, 0.2, 0.3, 0.5 and 0.6% of c.p.

The application was done in rotating pans, with a central disk inside and in the middle, also rotating, but in the opposite direction, for product distribution purposes. The device is known as “Rotary” (Seed Processing Holland®). After being treated and dried, the seed physiological and seed pathological issue were evaluated according to the following tests:

(i) Germination: Standard Germination Test (SGT) according to the Seeds Analysis Rules (ISTA, 2004; Brasil, 2009). Gerboxes were used, with two sheets of moistened germitest paper with 2.5 times their weight of distilate water, and four replicates (boxes) of 50 seeds, totaling 200 seeds per treatment. The boxes were placed in germination chamber at 20°C. The count of normal seedlings done on day 10 after sowing (DAS 10), with the value expressed in percentage;
ii) Germination Test First Count (GFC): the normal seedlings were counted on DAS 5, based on the SGT, with the value expressed in percentage;
iii) Length of shoot: in a random sample of ten seedlings evaluated on DAS 10 in SGT, the seedlings shoot length was measured with a ruler, and the value was expressed in cm;
(iv) Seedling dry matter weight: all normal seedlings evaluated on DAS 10 in SGT were placed in an oven with forced air circulation, at a temperature of 40°C, with subsequent weighting of total dry mass using analytical scale (0.1 mg accuracy). After dividing the value found by the number of seedlings, the amount of dry matter per plant was calculated in milligrams;
(v) Plug test: polypropylene trays were used to produce vegetable seedlings, containing 162 cells (31 cm^3 per cell) with Tropstrato® substrate, a pathogen free substrate, kept in a greenhouse during the assessment. Four replicates of 50 seeds per treatment were used. The emergence assessment was made on DAS 5 and DAS 10. Seedlings were considered emerged when cotyledons were fully open.
For the purposes of these seed physiological quality analyses, the experiments were fully random, with four replications always.
(vi) Seed pathology analyses: It was employed the blotter test, which consisted in distributing 25 seeds over three sheets of moistened filter paper previously prepared on petri dishes. Eight replications, totaling 200 seeds per treatment, were used. The plates were kept at 20 ± 2°C for a twelve-hour photoperiod under white fluorescent light during seven days. The seeds were evaluated individually under a magnifier, with the results expressed in percentage of seeds with fungus.

For seed pathology analyses, the results achieved for each fungus were processed separately in arc sin $\sqrt{(x/100)}$ to have the statistical analysis performed. The data obtained for all traits were subjected to variance analysis and the averages were compared by Tukey test at 5% probability.

RESULTS AND DISCUSSION

The seeds physiological quality test results may be found in Table 1. There was no interaction between the factors (fungicide lots and doses) in all variables considered, indicating independence between them. Regarding lots, no differences in total germination was found, with a 98% average (Table 1), that is much higher than the minimum standard allowed for marketing in Brazil by the Ministry of Agriculture, Livestock and Supply (MAPA), which is 75%. Also, no differences were found for the standard

Table 1. Germination (G), first count (GFC) in standard germination test, seedling length (SL), seedling dry matter (SDM), and emergence on DAS 5 (Em5DAS) and DAS 10 (Em10DAS) after sowing in substrate/tray with the three lots of Avenger hybrid broccoli seeds.

Lots	G (%)	GFC (%)	SL (cm)	SDM (mg)	Em5DAS (%)	Em10DAS (%)
82744	99 [a1]	98 [a]	4.9 [a]	4.5 [a]	92 [b]	97.4 [ab]
82745	97 [a]	96 [a]	4.7 [a]	3.0 [b]	93 [b]	95.4 [b]
82749	98 [a]	97 [a]	5.2 [a]	4.6 [a]	99 [a]	99.3 [a]
F_{lotes}	0.18 [ns]	2.22 [ns]	0.15 [ns]	204.85 [**]	10.67 [**]	4.51 [*]

[1] Averages followed by the same letter in the column do not differ by Tukey test at 5% probability. [ns] = non-significant by F test at 5% probability. [*] and [**] = significant by F test at 5% and 1% probability, respectively.

Table 2. Germination (G), first count (GFC) in standard germination test, seedling length (SL), seedling dry matter (SDM), and emergence on DAS 5 (Em5DAS) and DAS 10 (Em10DAS) after sowing in substrate/tray with the three lots of Avenger hybrid broccoli seeds, in different doses of Carboxin + Thiram during treatment.

Doses of Carboxin + Thiram	G (%)	GFC (%)	SL (cm)	SDM (mg)	Em5DAS (%)	Em10DAS (%)
0.0	98 [a1]	97 [a]	4.5 [a]	4.1 [a]	94 [a]	97 [a]
0.2	97 [a]	97 [a]	5.0 [a]	4.1 [a]	95 [a]	95 [a]
0.3	99 [a]	98 [a]	5.2 [a]	4.1 [a]	92 [a]	95 [a]
0.5	98 [a]	97 [a]	5.2 [a]	4.1 [a]	97 [a]	95 [a]
0.6	98 [a]	96 [a]	4.8 [a]	4.2 [a]	96 [a]	99 [a]
F_{doses}	0.59 [ns]	0.95 [ns]	0.10 [ns]	0.32 [ns]	2.13 [ns]	1.15 [ns]

[1] Averages followed by the same letter in the column do not differ by Tukey test at 5% probability. [ns] = non-significant by F test at 5% probability. [*] and [**] = significant by F test at 5% and 1% probability, respectively.

germination first count, with a 97% average. The first count (DAS 5) of seeds is considered as vigor test, in which samples that germinate faster, with higher percentage of normal seedlings on that date, are considered to be the strongest (Marcos Filho, 2005; Baalbaki et al., 2009). The 97% average achieved demonstrates the high seed vigor of all lots.

Despite there was no difference in seedling length on DAS 10 in SGT, with an average of 4.9 cm (Table 1), a lower seedling dry weight was observed in this same assessment (DAS 10) on lot 82745. This smaller vigor of this lot was confirmed in the emergence test, both on DAS 5 and DAS 10. Lot 82749 showed higher emergence in substrate on DAS 5 than the other two lots. Despite the small differences observed, with the highest vigor for lot 82749 and the lowest for lot 82745, the emergence test figures achieved were high for all lots, with a minimum of 92% on DAS 5 and 95% on DAS 10.

Some authors relate seed vigor to seedling production. Franzin et al. (2005) concluded that seed lots with higher initial quality, as detected by laboratory germination and vigor tests, produced seedlings with greater weight. The same was observed by Rodo and Marcos Filho (2003) in onion. However, in this study, the laboratory tests (germination and first count) were less sensitive than the "field" test, with production of seedlings in trays under uncontrolled temperature conditions. As to Carboxin + Thiram doses, there were no differences for all traits (Table 2), both by analysis of variance (F test) and by Tukey test, and both at 5% probability, which demonstrates that fungicide did not affect the seed quality, regardless of lot. Bittencourt et al. (2007) found no phytotoxic effect of this fungicide on peanut seed, and observed a greater emergence of treated seeds against the untreated control, because the fungicides reduced the incidence of "damping-off" caused by fungi in seeds and in the soil. Similar results were reported by Arsego et al. (2006) and Lobo (2008) with rice, and by Tropaldi et al. (2010) and Santos et al. (2012) with castor seed treated with this fungicide. Despite no phytotoxicity has been observed during treatment with the fungicide, the treated seeds in this study, regardless of dose, did not differ from untreated control for both in germination and emergence in plug test, probably because (a) it was observed that the presence of pathogens in seeds was low in all treatments, including the control (Tables 3 and 4), (b) a pathogen-free commercial substrate was used in the plug test, and (c) seed treatment with this fungicide does not

Table 3. Incidence of pathogenic and saprophytic fungal species in three lots of Avenger hybrid broccoli seeds subjected to treatment with Carboxin + Thiram.

Lots	*Alternaria* spp.	*Fusarium* spp.	*Penicillium* spp.	*Aspergillus* spp.	*Trichoderma* spp.	*Rhizopus* spp.
82744	0.6 [a1]	15.7 [a]	7.8 [b]	1.4 [a]	3.6 [a]	2.7 [a]
82745	0.0 [a]	11.1 [ab]	2.3 [c]	0.0 [a]	0.0 [b]	0.6 [a]
82749	0.6 [a]	6.4 [b]	14.3 [a]	0.0 [a]	0.6 [ab]	0.0 [a]
F_{lots}	0.50[ns]	1.37 [ns]	1.50 [ns]	0.76 [ns]	1.71 [ns]	1.23 [ns]

[1] Averages followed by the same letter in the column do not differ by Tukey test at 5% of probability.[ns] = non-significant by F test at 5% of probability.

Table 4. Incidence of pathogenic and saprophytic fungal species in Avenger hybrid broccoli seeds subjected to treatment with different doses of Carboxin + Thiram.

Doses of Carboxin + Thiram	*Alternaria* spp.	*Fusarium* spp.	*Penicillium* spp.	*Aspergillus* spp.	*Trichoderma* spp.	*Rhizopus* spp.
0.0%	1.9 [a1]	12.8 [a]	9.4 [a]	1.4 [a]	4.6 [a]	2.7 [a]
0.2%	0.0 [a]	12.8 [a]	6.2 [a]	0.0 [a]	0.0 [a]	0.0 [a]
0.3%	0.0 [a]	10.0 [a]	8.9 [a]	0.0 [a]	0.0 [a]	1.9 [a]
0.5%	0.0 [a]	11.7 [a]	10.5 [a]	1.0 [a]	0.0 [a]	0.0 [a]
0.6%	0.0 [a]	8.1 [a]	5.6 [a]	0.0 [a]	1.9 [a]	1.0 [a]
F_{doses}	0.50[ns]	1.37 [ns]	1.50 [ns]	0.76 [ns]	1.71 [ns]	1.23 [ns]

[1] Averages followed by the same letter in the column do not differ by Tukey test at 5% of probability.[ns] = non-significant by F test at 5% of probability.

interfere in the germination and vigor. Pinto (1998) found no difference in the emergence of treated and untreated sorghum seeds in sterilized soil, but noted higher emergence in seeds treated in unsterilized soil. Unlike the "large crops", it is a routine in technified systems for most vegetables, including broccoli, to have their seedling production in specific trays with fungi-free substrates (Minami, 2010).

Working with wheat crop, Marini et al. (2011) reported a reduction in germination and vigor of seeds treated with Carboxin+Thiram. Faria et al. (2003) observed that although there was an increase in germination and emergence of cotton seeds treated with Carboxin + Thiram, the seedlings were smaller and with less dry matter. Morales et al. (2012) observed higher vigor of rice seeds treated with this fungicide after seed storage for 60 and 90 days, compared to control. In all the works mentioned, the doses used were within the recommended range for each species, showing the importance of the study of different species, as results may be not equal. Furthermore, the sensitivity to fungicide treatment may vary, depending on the initial seed vigor (Lobo, 2008; Menten and Moraes, 2010). In this work, despite the difference in vigor between lots, the lots were not affected by treatment with fungicide, regardless of dose. Furthermore, as small quantities of fungicide per unit area are used, there is less risk of environmental pollution compared to foliar application.

As to seeds pathology (Tables 3 and 4), the pathogens *Alternaria* spp. and *Fusarium* spp. were detected, in addition to saprophytic species like *Penicillium*, *Aspergillus*, *Trichoderma* and *Rhizopus*. The higher incidence of *Fusarium* spp. was noticed in lot 82744, and the lowest in lot 82749. The incidence of *Alternaria* spp. was low, with no difference between lots and between doses; this fungus was only detected in seeds that were not treated with the fungicide, showing the effectiveness of treatment. Among the saprophytic fungi, as to *Penicillium* spp., all lots differed, being lot 82479 (Table 3) the most contaminated. However, there was no significant difference between the doses tested (Table 4). As to the other fungi, the general incidence was low and there was no difference between lots and doses used.

Conclusions

It is concluded that lot 82749 showed greater vigor than lot 82745, and the seed treatment with Carboxin + Thiram in the evaluated doses did not affect the physiological quality of seeds, so all doses tested (0 to 0.12% of p.a.) can be used. The incidence of fungi was very low in all lots, showing their good seed pathology quality.

CONFLICT OF INTERESTS

The author(s) have not declared any conflict of interests.

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Full Length Research Paper

Micropropagation of *Plectranthus edulis* (Vatke) Agnew from shoot tip and nodal explants

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Plectranthus edulis **Vatke belongs to the family of Lamiaceae, which occurs both as a wild and cultivated species. The major constraint in the cultivation of *P. edulis* is its low productivity due to shortage of planting materials and incidence of pests and diseases. In this study, an efficient protocol was established for the micropropagation of *P. edulis* germplasm using shoot tip and nodal explants. Explants were sterilized using different concentrations of Sodium hypochlorite (NaOCl) for different times of exposure. MS medium supplemented with different types and concentrations of auxin and cytokinin were used for culture initiation, shoot multiplication and root induction. NaOCl at a concentration of 2% and exposure time of 5 min gave 74.50±0.5% of clean culture for nodal and 69.83±0.76 from shoot tip. Six-Benzylaminopurine at 1.5 µM was found to be an optimum concentration for shoot induction, yielding 91.67±0.58% for nodal and 85.57±0.51% for shoot tip explants 3 weeks after culture. The combination of 2.0 µM BAP with 1.0 µM IAA was found to be the optimum concentration yielding 10.28±0.06 and 6.12±0.01 shoots per explants for nodal and shoot tip, respectively for shoot multiplication. Half strength MS medium with 2.0 µM IBA and 1.0 µM NAA gave the highest rooting percentage (97.00±0.28) with optimum root number (33) and length (3 cm). Up on acclimatization and transplanting, 83% survival efficiency was observed on soil mix ratio of 2:1:1 decomposed coffee husk, forest soil and sand, respectively. There were no observable variations with respect to morphology and growth characteristics to the greenhouse raised parent plants. The results obtained in this study permit the development of mass propagation protocol that could enable large scale commercial production of this highly demanded cultivar true-to type and provide a possible system towards genetic improvement of the crop.**

Key words: Explants, micropropagation, nodal culture, microshoots, plant growth regulators, plantlet.

INTRODUCTION

Plectranthus edulis Vatke is a tuber crop plant belongs to the family of Lamiaceae, in which the genus *Coleus* consists of over 350 tuber bearing and non-tuber bearing species. Although the origin of *P. edulis* was from Ethiopia, currently, it is widely distributed in Asia, Australia and other African countries (Codd, 1985), growing in mid and high altitude areas ranging from 1880 to 2200 m above sea level (Demissie, 1991; Greenway, 1944; Ryding, 2000).

P. edulis was a major traditional food crop for the rural

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communities of southwest Ethiopia. Nowadays, the potential uses of this indigenous crop have become deteriorated and the natural populations are rapidly disappearing as a result of the shortage of planting materials and attack by pests and microbial diseases (Ryding, 2000; Pratibha et al., 2011) to use for conventional propagation methods by tubers and stem cuttings.

Although several protocols have been developed for the micrpropagation of the species of genus Coleus such as *Plectranthus forskohlii* (Reddy et al., 2001; Praveena et al., 2012), *Plectranthus Blumei* (Smith and Murashige, 1982; Rani et al., 2006; Gaurav et al., 2010; Pratibha et al., 2011). *In vitro* propagation of *P. edulis* has recently been reported by Tsegaw and Feyisaa (2014) using meristem culture but there was no studies reported on the use of nodal and shoot tip explants and their sterilization experiments to avoid contamination yet. Therefore, there is need to develop plant tissue culture techniques for this species. One of the most important application of tissue culture as a tool of biotechnology is its application in further genetic improvement of the species and in the production of disease free plant materials. Besides, it enables production of large number of plantlets in a short period of time as well as conservation of germplasm under controlled conditions in small spaces with reduced labor requirement (Abraham, 2009). Moreover, each variety requires its own regeneration protocol (Gonzalez et al., 1999). The aim of this study was to develop a micropropagation protocol for *P. edulis* using shoot tip and nodal explants.

MATERIALS AND METHODS

Plant material

Healthy tubers of *P. edulis* were obtained from the Institute of Biodiversity Conservation (IBC), located in Addis Ababa (the capital city of Ethiopia), planted in a tin pot contained a sterilized soil that had a mixture of loam soil, coffee husk and sand (2:1:1, respectively), kept and grown under greenhouse condition of the College of Natural Science, Jimma university until used for experiment. The growing plant materials were daily watered with tap water and sprayed with 0.3% Mancozeb at 15 days interval to control fungal infection. Two months old, healthy and vigorous plants were used as a source of explants.

Culture medium and growth regulators stock preparation

MS media was prepared by dissolving 4405.19 gl^{-1} of the readily available medium with vitamins (company name) with sucrose (30 g) in double distilled water. The pH of the solution was adjusted to 5.7 to 5.8 using 0.1 N HCl or 1N KOH before making up to final volume. For solidification, agar powder (Company name) 0.8% (w/v) was added to the moderately warm solution and then, melted by constant stirring. The medium (25 ml) was dispensed into borosilicate test tubes, plugged with cotton, covered with aluminum foil and autoclaved at 121°C for 20 min in a vertical autoclave. Different growth regulators viz. 6-benzylamino purine (BAP), Kinetin, α-naphthalene acetic acid (NAA), and indol-3-butyric acid as stock solutions of 1 mg/L and required amount for specific concentrations were added to the medium before autoclaving.

Explant preparation and sterilization

Healthy and juvenile plants were taken from mother stock *P. edulis* maintained in greenhouse. Both shoot tip (1 to 1.5 cm long) and nodal explants (1.5 to 2 cm long) were excised and sterilized with different concentrations of NaOCl for different times of exposure, washed three times with tap water and detergent using sponge. Thereafter, the explants were kept under running water for 10 min and finally washed with double distilled water.

Culture initiation

Sterilized explants were cultured on agar solidified (0.8% agar-agar) full strength MS basal medium supplemented with different concentrations of BAP (0, 0.5, 1.0, 1.5, and 2.0 mg/L) and Kinetin (0, 1.0, 2.0, and 3.0 mg/L) to be tested for shoot induction rate of shoot tip and nodal explants in CRD design in 4x3x2 factorial combinations.

Shoot multiplication

Shoot buds initiated from those explants that had responded well to the prevailing culture conditions were transferred singly onto a shoot multiplication MS medium containing different concentration and combination of BAP and NAA. The experiment was thus being arranged in a 4x3x2 factorial in CRD. After two-three weeks, cultures proliferating shoot clumps were divided and sub cultured on to a fresh medium of similar composition.

Root induction

Well-developed microshoots obtained from shoot multiplication media were transferred for rooting on agar solidified (0.8% agar-agar) half strength MS basal medium was supplemented with 3% sucrose and different concentrations of IBA and NAA. The experiment was laid with treatment of five concentrations for IBA (0, 0.5, 1.0, 1.5, 2.0 and 2.5 mg/L) and three concentrations for NAA (0, 0.5, 1, and 1.5 mg/L) auxins in CRD in 5 x 3 factorial combination.

Acclimatization

Plantlets with well developed from rooting media were isolated, washed and then treated in light polyethylene pot covered by 70% shade net above it. The system was designed to give high humidity (80 to 90%) to prevent desiccation for ten days, prior to their transfer to a shade house. Starting from the 15^{th} day, the RH within the system was reduced to gradually to 60% at the end of the month. After the month, the plantlets were transferred to a 70% shade net, where they were retained for a month. Later they were transferred to a 30% shade net and maintained there for three weeks. The numbers of survived plantlets were recorded in each step.

Experimental design and treatments

All experiments were laid in a Completely Randomized Design (CRD) with factorial treatment combinations, having three replications per treatment and five explants per jar under each

replicate. All the experiments were repeated two times to ensure reproducibility of the results and the average of these two were considered for analysis. Prior to laying the multiplication and rooting experiments, sufficient explants were made to multiply till the desired numbers of explants are generated. At all times, explants were cultured on a PGR-free medium prior to their use for an experiment; so as to avoid any sort of carryover effects from the previous culture medium they were retained. Controls were set for each experiment with zero concentration of the analyses considered.

Data recording

After sterilization experiment, the number and percentage of explants affected by contamination and tissue death was recorded during the first two weeks of culture for shoot tip and nodal explants independently. For the second experiment, the number and/or percentage of explants forming shoot buds was recorded after four weeks for shoot tip and nodal explants independently. Number of shoots proliferated from each shoot bud on multiplication media was counted at three weeks interval during sub culturing. The number of roots (including the main roots and their branches), shoot length and the length of the roots was recorded after three weeks of culture for experiment four.

Data analysis

Average of the data collected from the two repetitions for each experiment were independently subjected to statistical analysis using the SAS statistical software (version 9.2) and ANOVA was constructed, followed by mean separation using appropriate procedures (REGWQ). When ANOVA indicated significant treatment effects (5, 1 or 0.1%) based on the F-test, probability level of 0.05 ($p \leq 0.05$) was considered to determine which treatments were statistically different from the other treatments.

RESULTS AND DISCUSSION

Effects of NaOCl concentrations and exposure time on sterilization of *P. edulis* explants

The analysis of variance showed that the concentration of active chlorine in sodium hypochlorite solution, time duration of explants exposure to the sterilants and interaction of concentrations to time duration had very highly significant effect ($p < 0.0001$) on both of contamination and clean culture of shoot tip and nodal explants .Very highly significant difference had also been revealed between the two types of explants (treatment * explants = $p < 0.0001$) indicating that the level of contamination and clean culture was influenced by the concentration of NaOCl and duration of exposure time and the mean average value for contamination and clean culture of node exceeded shoot tip. The highest rate of clean culture (69.83±0.76%) was obtained from treatment concentration of 1% active chlorine (in NaOCl solution) with five minute exposure duration for shoot tip explants. For nodal explants, the same percentage concentration and time exposure duration was found to be the most effective treatment combination with mean average result of 74.50±0.50 clean culture (Table 1). The highest percentage of contamination were observed from nodal and shoot tip explants due to the low concentration NaOCl (0.5%) at short exposure of time (three and five minute) for both types of explants. In the higher concentration of sodium hypochlorite (2% NaOCl) and long exposure of time (seven and nine minutes), percentage of contamination was very low but tissue death is highest.

In the present study, one percent sodium hypochlorite treatment of five minutes exposure of time was optimum for sterilization of *P. edulis* explants (Table 1). Different scholars use mercuric chloride for explant surface sterilization (Bhattacharya and Bhattacharya, 2001; Rani et al., 2006) but using mercuric chloride for surface sterilization is not environmentally friendly so that it is not recommendable.

Effect of different concentration and combination of BAP and Kinetin shoot initiation on nodal and shoot tip explants of *P. edulis*

Aseptic shoot tips and nodal cultures were transferred on MS media fortified with different concentrations of BAP in combination with kinetin for four weeks to determine optimum medium for shoot induction of *P. edulis*. The analysis of variance (Table 2) showed that the interaction with BAP and type of explants had very highly significant effect ($p< 0.0001$) on the shoot induction rate. Interaction effect of explants type with BAP on rate of shoot induction was found to be highly significant (BAP*Explant).The response of shoot tip and nodal explants to a given concentration of BAP was not the same that the nodal explants gave greater response than shoot tip explants (Table 2).

The highest rate of shoot induction (91.67±0.58%) was achieved on MS medium supplemented with 1.5 mg/L BAP from nodal and 85.57±0.5% on 1.5 mg/L BAP from shoot tip on MS media (Table 2). For both shoot tip and nodal explants, MS basal media added with 1.5 mg/L BAP used alone were found to be optimum media for *in vitro* shoot initiation of *P. edulis*.

In combination of BAP and Kinetin, the highest percentage of induction was observed at 1.5 mg/L BAP and 3.0 mg/L Kinetin (73.20±1.05% and 70.56±1.39%) for nodal and shoots tip explants, respectively (Table 2). From the given concentrations, high concentration of BAP and kinetin and a medium with free growth regulator resulted in low percentage of shoot induction. Therefore, BAP proved to be a more effective than Kinetin for multiple shoot induction of *P. edulis.* The present result was in accordance with the result of Vasile et al. (2006) achieved high regeneration shoot induction from *P. blumei*, using 1.5 mg/L of BAP. Similar result was also reported by Pratibha et al. (2011) that 1.5 mg/L BAP was found to be optimum for shoot initiation. High BAP

Table 1. Interaction effect of Sodium hypochlorite concentrations and its time of exposure on sterilization offshoot tip and nodal explants of *P. edulis.*

Conc. NaOCl	Duration (min)	% contamination		% Clean culture		% Tissue death	
		Nodal (Mean ±StdDev)	Shoot tip (Mean ±StdDev)	Nodal (Mean ±StdDev)	Shoot tip Mean ±StdDev	Nodal (Mean ±StdDev)	Shot tip Mean ±StdDev
0.5	3	89.76±0.68^{a}	87.73+0.64^{a}	10.24±0.65^{k}	12.27± 0.63^{i}	0.00±0.00^{n}	0.00±0.00^{l}
0.5	5	79.83±0.76^{b}	75.00±0.50^{b}	17.17±1.26^{j}	22.16±0.76^{g}	2.50±0.50^{m}	3.40±0.52^{k}
0.5	7	68.40±0.52^{c}	65.83±0.76^{c}	25.60±0.36^{h}	29.00±1.00^{f}	5.76±0.68^{l}	5.76±0.68^{j}
0.5	9	59.76+ 0.68^{d}	57.73+0.64^{d}	32.24+0.74^{g}	34.27+0.86^{e}	8.40+0.52^{kl}	8.50+0.50^{i}
1	3	49.93±0.90^{e}	48.76±0.68^{e}	40.07±1.10^{f}	42.00±1.00^{d}	10.60±0.52^{k}	9.40±0.52^{i}
1	5	15.83±0.76^{f}	20.83±0.76^{f}	74.00±0.40^{a}	69.16±1.89^{a}	11.83±0.76^{k}	10.43±0.51^{i}
1	7	13.90±0.85^{g}	18.60±0.52^{g}	60.10±1.15^{b}	62.00±1.00^{b}	21.43±0.51^{j}	19.73±0.64^{h}
1	9	12.40+ 0.52^{g}	16.43+0.51^{h}	54.00+0.80^{c}	53.57+0.51^{c}	29.73+0.64^{i}	29.76+0.68^{g}
1.5	3	10.66 ±0.76^{h}	13..83±0.76^{i}	51.34±1.32^{d}	40.00±1.00^{d}	37.83±0.76^{h}	47.73+0.64^{f}
1.5	5	10.60±0.52^{h}	10.60±0.52^{j}	43.40±1.96^{e}	29.40±1.21^{f}	55.90±0.85^{g}	59.83±0.76^{e}
1.5	7	8.40±0.52i	8.40±0.52^{k}	20.00±1.00^{i}	20.00±0.30^{gh}	69.76±0.68^{f}	72.50±0.50^{d}
1.5	9	6.43+0.51^{j}	6.43+ 0.51^{l}	18.33+1.04^{ij}	17.57+1.24^{h}	76.50+0.50^{e}	75.76±0.68^{c}
2	3	5.60±0.52^{j}	5.83±0.76^{l}	9.40±0.76^{k}	10.00±0.50^{j}	85.83±0.76^{d}	75.43+0.51^{c}
2	5	3..83±0.76^{k}	2.60±0.52^{m}	7.17±0.64^{l}	7.40±0.52j^{k}	89.76±0.68^{c}	89.73±0.64^{b}
2	7	2.60+ 0.52^{k}	2.50+ 0.50	5.40+ 0.50^{l}	5.63+0.40k^{l}	92.60+0.52^{b}	92.40±0.52^{b}
2	9	00.00±0.00^{l}	0.00±0.00^{n}	2.00±0.20^{m}	3.00±0.70^{l}	98.73±0.64^{a}	96.40±0.52^{a}
CV		*2.37*	*2.18*	*3.15*	*3.69*	*1.96*	*2.0*

Means with the same letters in a column are not significantly different from each other by Ryan - Einot - Gabriel - Welsch Multiple Range Test (REGWQ) at α= 5%.

concentration decreased the shoot production either by inhibition of shoot initiation or by encouraging callusing (Figure 1).

Effect of different concentration and combination of BAP and NAA on shoot multiplication of *P. edulis*

Those shoot buds induced well on the prevailing shoot induction medium were transferred to MS media supplemented with BAP (1.0 to 3.0 mg/L) alone and in combination with (1 to 3 mg/L) NAA. Cultures were sub cultured twice and the effect of hormones on *in vitro* shoot multiplication of *P. edulis* cultivar was evaluated.

In this study, the significance of BAP and its interaction with NAA were considered. The ANOVA revealed that the concentration of BAP both alone and together with NAA had very highly significant effect ($p < 0.0001$) on shoot multiplication rate. Shoot buds raised from nodal explants responded exceeds shoot tip shoot multiplication and this indicating the significant effect of explants at this stage.

In this study, maximum number of shoot proliferation 10.28±0.06 and 6.12±0.01 was obtained on MS medium containing 2.0 mg/L BAP and 1.0 mg/L NAA from nodal and shoot tip, respectively (Table 3). Similar results were reported by Rani et al. (2006). Some of the previous studies, where BA and NAA were found to be useful in shoot multiplication from nodal segments and shoot tip explants of various other plants, e.g. *Jasminum officinale* (Bhattacharya, 1997), *Vanilla planifolia* (George and Ravishankar, 1997), *Aristolochia indica* (Manjula et al., 1997), *Vitex negundo* (Kannan and Jasrai, 1998), *Syzygium travancoricum* (Anand et al., 1999) and *Ancistrocladus abbreviatus* (Bringmann et al., 1999). However, in certain other *Plectranthus* species*, P. forskohlii* (Sen and Sharma, 1991) and *P. parviflorus* (Ponsamuel et al., 1994), BA (2 mg/L) alone was sufficient for formation of multiple shoots from nodal segments and shoot tips. Similarly, Hiregoudar et al. (2005) also reported that addition of BA (2 µM) alone to MS medium is responsible for shoot induction. In the present study, among all the combinations and concentrations, the longest shoots 4.51±0.04 cm and 3.45±0.09 cm were observed on the medium containing 2.0 mg/L BAP with 1.0 mg/L NAA for both nodal and shoot tip explants, respectively. A medium free growth regulators and a medium with high concentration of BAP alone and in combination with NAA resulted in low multiplication rate (Table 3). Length of shoots that were obtained from nodal explant was longer than observed from shoot tip explants.

Effect of different concentrations of IBA, and NAA for *in vitro* root initiation of *P. edulis*

The highest rooting percentage (97.00+ 0.28) was

Table 2. The effects of different concentrations of BAP and Kinetin alone and in combination on MS medium for percentage of shoot induction of *P. edulis* shoot tip and nodal explants.

Conc. of PGRS		Explant	
		Nodal	Shoot tip
BAP(mg/l)	Kin(mg/L)	Mean±STD	Mean±STD
0	0	10.56±0.51^{l}	9.60±0.52^{o}
0	1	40.92±0.88^{k}	35.62±1.07^{n}
0	2	41.87±0.26j^{k}	40.86±1.02^{m}
0	3	43.62±0.67^{j}	42.65±0.56^{m}
0.5	0	81.25±0.90^{c}	74.32±0.58^{c}
0.5	1	53.08±0.88^{i}	50.18±1.04^{l}
0.5	2	57.57±0.38^{h}	53.72±0.62^{k}
0.5	3	58.33±0.32^{h}	57.66±0.57^{j}
1	0	85.50±0.50^{b}	77.76±0.67^{b}
1	1	61.58±0.51^{g}	60.58±1.41^{hi}
1	2	64.78±1.07^{f}	62. 35±1.52^{hg}
1	3	66.67±1.15^{f}	63.56±1.25^{fg}
1.5	0	91.67±0.58^{a}	85.57±0.51^{a}
1.5	1	70.68±1.00^{e}	66.00±0.99^{ef}
1.5	2	71.47±1.04d^{e}	67.38±1.19^{e}
1.5	3	73.20±1.05^{d}	70.56±1.39^{d}
2	0	66.63±0.54^{f}	64.58±1.00^{fg}
2	1	61.25±1.56^{g}	59.68±0.58^{ij}
2	2	56.65±0.56^{h}	54.65±0.56^{k}
2	3	53.37±0.54^{i}	50.40±1.21^{l}
CV		1.34	1.70

Means within a column followed by the same letters are not statically significant at p< 0.01 by Ryan - Einot - Gabriel - Welsch Multiple Range Test (REGWQ).

(a) (b) (c) (d) (e)

Figure 1. Micropropagation of *P. edulis*; (a) Shoot induction on 1.5 mg/l BAP, (b) Shoot multiplication on 2.0 mg/l of BAP and 1.0 mg/l IAA, c) Rooting on half - strength medium with 2 mg/l IBA and 1.0 mg/l NAA, d) Plantlets ready to transfer on sterile soil mix. e) Acclimatization on 2:1:1 top soil, coffee husk and sand soil mix (f) plantlets under greenhouse condition 6 weeks after transfer.

obtained on half-strength MS medium at 2.0 mg/L of IBA followed by 85.25+0.97% at 1 mg/L of NAA (Table 4). Among the given concentrations auxins with higher concentration resulted in less rooting percentages. Naphthalene acetic acid (NAA) at a concentration of 1.5 mg/L resulted in less percentage of rooting (51.17±0.67) that was less than the root induced from all the rest at high and low concentration. The longest shoot (5.90±0.09 cm) was obtained from a medium that contained 2.0 mg/L 1BA followed by 3.32±0.24 cm from 1.0 mg/L of NAA. Smallest shoot height 2.00±0.23 cm were obtained from 2.5 mg/L of IBA.

The highest mean number 32.73±0.14 of roots were obtained from 2.0 mg/L of IBA followed by 19.32±0.71 on

Table 3. Effect of different concentrations and combinations of BAP and NAA treatments on shoot multiplication of *P. edulis.*

Levels of PGR		Nodal		Shoot tip	
BAP (mg/L)	NAA (mg/L)	Shoot number Mean ± StdDev	Shoot length Mean ± StdDev	Shoot number Mean ± StdDev	Shoot length Mean ± StdDev
0	0	2.41±0.01^{kl}	2.12±0.06^{i}	2.49±0.07^{gh}	1.99±0.01^{e}
0	1	2.70±0.10	2.96±0.07^{hi}	2.78±0.05^{e}	2.02±0.02^{e}
0	2	2.68±0.02^{fgh}	2.15±0.03^{hi}	2.51±0.01^{gh}	2.07±0.01^{de}
0	3	2.88±0.07^{d}	2.25±0.06g^{hi}	2.85±0.02^{cde}	2.13±0.02^{de}
1	0	2.54±0.06^{ijk}	2.97±0.03^{c}	2.43±0.03^{hij}	2.07±0.06^{de}
1	1	2.38±0.04^{l}	2.56±0.06^{e}	2.65±0.02^{f}	2.62±0.10^{c}
1	2	2.62±0.01	2.19±0.04^{ghi}	2.19±0.04^{m}	2.80±0.10^{bc}
1	3	2.52±0.06^{ijk}	2.75±0.07^{d}	2.31±0.01^{kl}	2.19±0.08^{de}
1.5	0	2.85±0.01^{de}	2.48±0.06^{ef}	2.80±0.04^{de}	2.91±0.06^{b}
1.5	1	2.55±0.01^{hijk}	2.51±0.04e	2.35±0.02^{jkl}	2.75±0.12^{bc}
1.5	2	2.46±0.06^{jkl}	3.18±0.05^{b}	2.53±0.03^{gh}	2.70±0.03^{c}
1.5	3	2.57±0.01^{ghij}	2.14±0.04^{hi}	2.91±0.01^{c}	2.15±0.04^{de}
2	0	3.35±0.05^{b}	2.30±0.10^{gh}	3.58±0.03^{b}	2.27±0.06^{d}
2	1	10.28±0.06^{a}	4.51±0.04^{a}	6.12±0.01^{a}	3.45±0.09^{a}
2	2	3.23±0.05^{c}	1.70±0.05^{j}	2.60±0.02^{gf}	2.92±0.02^{b}
2	3	3.18±0.06^{c}	2.21±0.04^{ghi}	2.90±0.05^{cd}	2.10±0.07^{de}
3	0	2.71±0.04^{f}	2.35±0.02^{fg}	2.25±0.06^{ml}	2.03±0.02^{e}
3	1	2.52±0.02^{ijk}	2.28±0.11^{ghi}	2.45±0.05^{hij}	2.17±0.09^{de}
3	2	2.75±0.02^{ef}	2.52±0.04^{e}	2.57±0.04^{gf}	2.15±0.08^{de}
3	3	2.45±0.04^{jkl}	2.89±0.05^{cd}	2.39±0.02^{ijk}	2.20±0.09^{de}
CV		1.58	2.36	1.38	2.98

Means within a column followed by the same letters are not statically significant at p< 0.01 by Ryan - Einot - Gabriel - Welsch Multiple Range Test (REGWQ).

Table 4. Effect of various concentrations of IBA and NAA on rooting of proliferated shoots of *P. edulis* cultured on half - strength MS medium.

Conc. of PGRs (µM)		Rooting (%) (Mean ± SD)	Shoot height (cm) (Mean ± SD)	Root number (Mean ± SD)	Root length (cm) (Mean ± SD)
IBA(mg/L)	NAA(mg/L)				
0	0	49.71±0.47^{n}	2.70±0.26^{h}	4.60±0.10^{m}	1.07±0.04^{jk}
0	0.5	70.95+0.39^{f}	3.17±0.22^{fgh}	8.30±0.43^{gh}	1.45±0.09^{ghi}
0	1	85.25+0.97^{c}	3.32±0. 24^{fg}	19.32±0.71^{e}	1.59±0.20^{fgh}
0	1.5	51.17±0.67^{mn}	2.71±0.14^{h}	4.83±0.20l^{m}	1.52±0.17^{ghi}
0.5	0	59.32±0.88^{i}	3.48±0.19^{ef}	7.80±0.26^{ghi}	2.11±0.14^{cde}
1	0	84.39+ 0.61^{c}	4.65±0.30^{bcd}	26.07±0.55^{c}	2.15±0.08^{bcd}
1.5	0	90.76+1.11^{b}	4.90±0.18^{b}	30.57±0.24^{b}	2.47±0.04^{b}
2	0	97.00+ 0.28^{a}	5.90±0.09^{a}	32.73±0.14^{a}	2.95±0.08^{a}
2.5	0	68.55±0.71^{g}	2.60±0.07^{i}	17.37±0.01^{f}	1.75±0.01^{efg}
CV		0.99	5.53	2.19	6.03

(±). Means within a column followed by the same letters are not statically significant at α= 5 % by Ryan - Einot - Gabriel - Welsch Multiple Range Test (REGWQ).

1.0 mg/L NAA. Highest concentrations of auxins resulted in less number of root. Relatively, less number of roots 2.60±0.07 was obtained from IBA at 2.5 mg/L (Table 4). Similar results were reported by Rani et al. (2006) observed that 1/2-MS with 2 mg/L IBA was found to be the best treatment for induction of roots. Root induction decreased with increase in concentration of IBA. NAA resulted in comparatively lesser number of roots. In this

study, half - strength MS medium supplemented with IBA (0.5 1.0, 1.5, 2.0 and 2.5 mg/L) and NAA (0.5 1.0 and, 1.5 mg/L) were evaluated and relatively 2.0 and 1.0 mg/L IBA and NAA respectively gave good rooting percentage. The concentrations beyond these led to a decrease in the number of roots and root length per rooted explant and rooting rate. Similar findings on some other plants, e.g. *Elaeagnus angustifolia* (Iriondo et al., 1995), *Asparagus robustus* (Nayak and Sen 1998), *Eucalyptus tereticornis* (Sharma and Ramamurthy, 2000) and *Hemides musindicus* (Sreekumar et al., 2000).The root elongation phase is very sensitive to auxin concentration, and it is inhibited by high concentration of auxin in the rooting medium. Daffalla et al. (2011) reported that roots may require a less concentration of auxin to grow, but root growth is strongly inhibited by its higher level because at this level, auxin induces the production of ethylene, a root growth inhibitor.

Acclimatization *in vitro* derived *P. edulis* plantlet

The establishment of *in vitro* plantlets under different environmental conditions was greatly affected in terms of survival percentage of plantlets. In the present study, the plantlets showed 83.4% survival efficiency. The plantlets transferred under net house conditions resulted in the best establishment, whereas no plantlets could be established under direct field conditions (Figure 1).

Conclusion

One percent concentration of NaOCl solution for five minute exposure time were found to be optimum treatment for sterilization of shoot tip and nodal explants of *P. edulis*. The maximum percentage of shoot induction (91.67±0.58) and (85.57±0.51) was observed on an MS medium supplemented with 1.5 mg/L BAP from nodal and shoot tip explants respectively. MS basal medium supplemented with 2.0 mg/L BAP and 1.0 mg/L NAA resulted in 10.28±0.06 shoot number with best and vigor morphological appearance. Best rooting percentage was achieved on half strength MS basal media containing 2.0 mg/L IBA which resulted mean values of 97.00+ 0.28 with 32.73±0.14 root number, followed by half strength MS basal media containing 1 mg/L NAA which resulted 85.25+0.97 with 19.32±0.71 root number, 1.59±0.20 cm root length and 3.32±0.24 shoot length. Those plantlets well performed *in vitro* showed 85% survival efficiency after hardening and acclimatization on soil mix ratio of 2:1:1 decomposed coffee husk, top forest soil and sand respectively.

Conflict of Interest

The authors have not declared any conflict of interest.

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Effect of base saturation and nitrogen dose on cultivation of crambe

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The grain production potential of crambe reported in literature may vary from 1000 to 1500 kg ha^{-1}, but there are not yet any recommendations regarding specific nitrogen (N) fertilization for this crop. The objective of this study was to evaluate the effect of base saturation and the addition of N doses on plant development and productivity of crambe. This study was developed at the São Tomaz Jatobá farm in the municipality of Rio Verde, GO in a Distroferric Red Latosol. The experiment consisted of a factorial (4×3) design with four repetitions totaling 48 plots distributed in random blocks. Four levels of base saturation (V%) were evaluated as follows: 34 (natural soil), 40, 50 and 60. Moreover, the following three N doses were evaluated: Control (without application de N), 40 kg ha^{-1} N and 80 kg ha^{-1} N. The experimental plots were rectangular and measured 9 m^2 with five planted lines and a spacing of 0.45 m between rows. Planting was performed on March 8th, 2011 using the FMS Brilhante cultivar. The following variables were evaluated: Root dry mass and shoot dry mass in three distinct periods (35, 45 and 55 days after emergence); grain yield; and oil content. The addition of the N doses increased the root dry masses, shoot dry masses and yield, but N addition did not influence oil content. In general, the best N dose was 40 kg ha^{-1}. Base saturation linearly or quadratically influenced all parameters evaluated, and the best base saturation observed in this study was 50%.

Key words: FMS Brilhante cultivar, nitrogen fertilization, productivity, oil content, Brazil.

INTRODUCTION

Several plants have been studied with the objective of providing oil for biodiesel production. Among these plants, crambe (*Crambe abyssinica*) is notable. Crambe is a winter crop and can be grown late in the season during periods in which risks for other crops in the late off-season would be high in the Midwest region of Brazil (Pitol et al., 2010). For these reasons, this plant has attracted interest as an alternative for the off-season and crop rotation (Panno and Prior, 2009). One of the main characteristics of crambe cultivation is its earliness, producing mature grains at 90 to 100 days with uniform maturation, which facilitates mechanical harvesting

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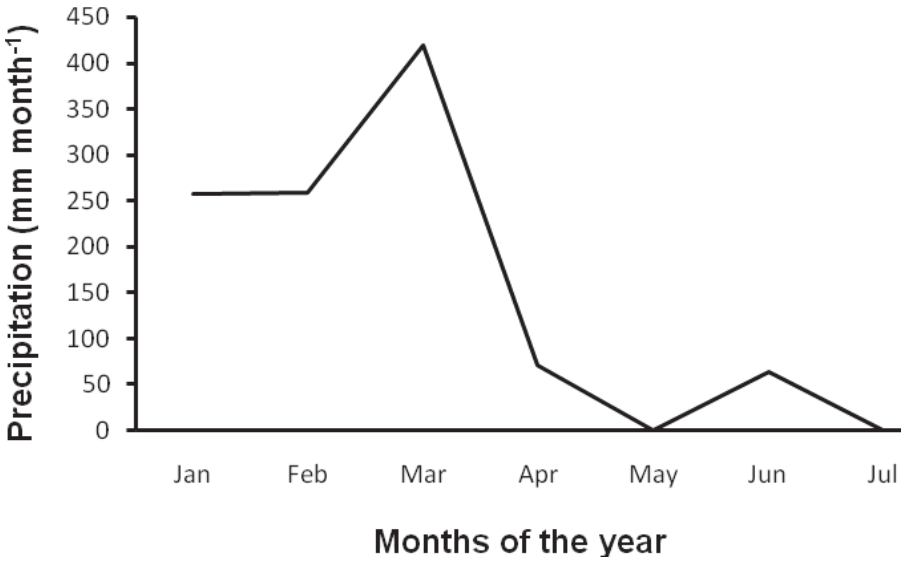

Figure 1. Accumulated precipitation (mm month^{-1}) during development of the crambe crop in the municipality of Rio Verde, GO during the year of 2011.

(Lazzeri et al., 1995; Falasca et al., 2010). Jasper et al. (2010a) reported that the crambe culture has lower production costs than other oil crops, such as canola, sunflower and soybean. According to Heinz et al. (2011), crambe litter presents greater persistence than other crops used as ground cover, and potassium (K), phosphorus (P) and magnesium (Mg) are the nutrients most rapidly released for the subsequent culture. The potential yield of crambe reported in literature varies from 1000 to 1500 kg ha^{-1} (Pitol et al., 2010; Rogério et al., 2012; Santos et al., 2012). Regarding oil quality, data indicates an average production of 38% for total oil content, and this rate may vary according to the climate and soil conditions (Silva et al., 2011). The oil is composed of approximately 57% erucic acid, which can cause liver and kidney damage as well as loss of appetite in monogastric animals cited by Carlsson et al. (2007).

Regarding soil fertility, there are no specific recommendations of nitrogen (N) doses for the crop, and only a few studies on this subject have been published. In plants, mineral N is absorbed in the nitrate or ammonium forms, and it preferably comes into contact with the roots by mass flow (Malavolta et al., 1997). N makes up amino acids and nucleotides, and it is the primary nutrient for obtaining high yields in annual cultures (Castro et al., 1999). According to Soratto et al. (2013), N is the nutrient most exported in crambe production under field conditions reaching 54 kg ha^{-1} N. According to the results obtained by Souza et al. (2009), the crude protein obtained in crambe cake (31.7%) indicates crambe crops demand N under high productivity conditions. Therefore, it is important to know the response potential of the crambe crop to this nutrient to enable more environmentally and financially efficient fertilization.

Considering base saturation, reports have suggested that the crambe culture develops better and achieves better grain yields in eutrophic soils (Broch and Roscoe, 2010). According to these authors, crambe is quite tolerant to water stress, but this tolerance is directly linked to its deep rooting ability, which in turn depends on a corrected soil profile for acidity and aluminum toxicity. According to Janegitz et al. (2010), base saturation suitable for crambe development and production in medium textured soils is between 50 and 65%. Broch and Roscoe (2010) stated that the conditions of soil acidity used for crambe production are the same as the main summer crops. Only a few results are available on the ideal level of base saturation for good development of crambe. Thus, it is important to obtain information on the development of this crop in different levels of soil base saturation and in different weather conditions.

The objective of this study was to evaluate the effect of increasing soil base saturation and addition of N on vegetative growth, grain yield and oil content of crambe.

MATERIALS AND METHODS

This study was performed at the São Tomaz Jatobá farm (17° 49' 22.63'' S and 50° 56' 21.87'' W; elevation of 725 m) in the municipality of Rio Verde, GO in Brazil. The area was being cultivated in a succession of soybeans and maize where soybean was the culture used in the previous season (2010/2011), which was harvested in January 2011. According to the Köppen classification, the climate in the region is Aw, which is defined as humid tropical with a rainy season in the summer and dry in the winter. The annual average temperature varies between 20 and 35°C, and the annual precipitation ranges from 1,500 to 1,800 mm. Intensities of precipitation occurring during culture development are shown in Figure 1, and the average temperature is shown in Figure 2. Temperature and precipitation data were obtained from the meteorological station located in the University Campus of Fesurv

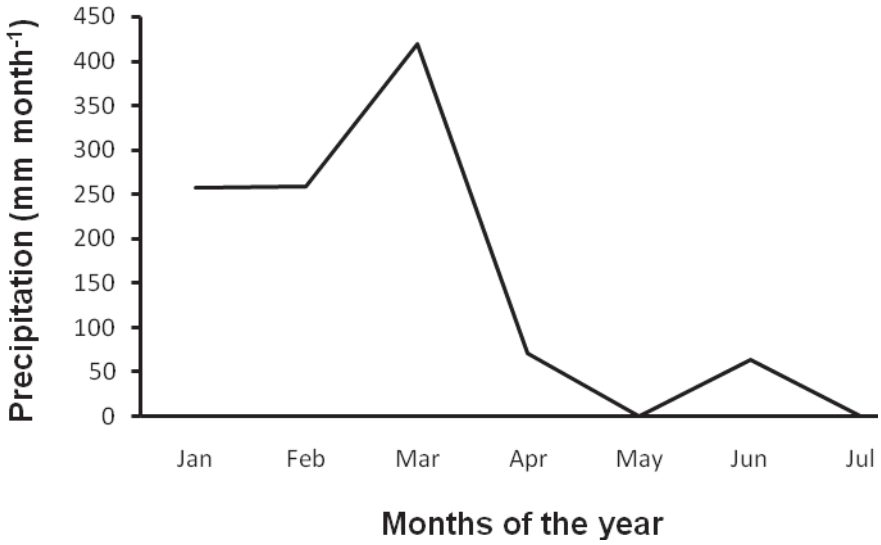

Figure 2. Average monthly temperature (°C) during crambe crop growth in the municipality of Rio Verde, GO during the year of 2011.

(University of Rio Verde) in Rio Verde, GO. The average monthly temperature (°C) was also monitored during crambe growth, as shown in Figure 2.

The undulated relief (8% slope) and soil classified as Distroferric Red Latosol (Embrapa, 2006) presented the following chemical and textural characteristics at depths of 0 to 20 cm: pH ($CaCl_2$) = 4.7; calcium (Ca), Mg, K, aluminum (Al) and cation exchange capacity (CEC) values of 1.7, 0.6, 0.10, 0.06 and 7.1 $cmol_c$ dm^{-3}, respectively; $P_{(mel)}$, sulfur (S), zinc (Zn), boron (B), copper (Cu) and manganese (Mn) contents of 5.5, 11.3, 1.8, 0.12, 1.5 and 41.6 mg dm^{-3}; base saturation (V%) = 33.8; organic matter (OM) = 2.0 g dm^{-3}; and clay, silt and sand contents of 350, 50 and 600 g kg^{-1}, respectively. For depths of 20 to 40 cm, the soil analysis results presented the following characteristics: pH ($CaCl_2$) = 4.4; Ca, Mg, K, Al and CEC values of 0.9, 0.3, 0.07, 0.23 and 6.0 $cmol_c$ dm^{-3}, respectively; $P_{(mel)}$, S, Zn, B, Cu and Mn contents of 1.9, 33.7, 0.4, 0.12, 1.9 and 21.9 mg dm^{-3}; V% = 21.3; OM = 15.2 g dm^{-3}; and clay, silt and sand contents of 375, 50 and 575 g kg^{-1}, respectively.

The experiment was arranged in a factorial design (4 × 3) with four levels of base saturation and three N doses. The levels of base saturation were as follows: 34 (natural soil), 40, 50 and 60%. Moreover, the following N doses were evaluated using urea as a source: Control (without application de N), 40 kg ha^{-1} and 80 kg ha^{-1}. Four replications were used for each treatment totaling 48 plots. Natural base saturation of the soil was elevated with application of lime, and the need for correction (NC) was calculated using the following formula for increased base saturation. A lime filler (30.5% calcium oxide (CaO) and 18.7% magnesium oxide (MgO)) was applied to the soil. Application and incorporation into the soil in each plot occurred 15 days before planting. In all plots, a basic fertilization was performed consisting of 80 kg ha^{-1} phosphorus pentoxide (P_2O_5) used as source of single super phosphate applied with the seeds at planting and 50 kg ha^{-1} potassium oxide (K_2O) used as a source of potassium chloride (KCl) applied 22 days after emergence (DAE) in coverage. A row was dug approximately 3 to 4 cm deep at a distance of approximately 15 cm from the planting row where all N and K was deposited after which it was immediately covered by the removed soil.

The experimental plots were in the shape of a rectangle measuring 9 m^2 (2.25 × 4 m). Each plot consisted of five planted rows that were 4 m long, with 0.45 m spacing between rows. The distance between the experimental plots was 1 m, and the distance between blocks was 2 m. Desiccation of the experimental area was performed one day after planting using glyphosate at a dose of 3 L ha^{-1} in association with carfentrazone at a dose of 50 ml ha^{-1}.

Planting was conducted on March 8th, 2011 using a SHM 11/13 (planter brand Semeato) adapted for a spacing of 0.45 m using a cutting disc. The planting density was 12 kg ha^{-1} of seeds at a depth of 2 cm. The crambe seeds used were from the FMS Brilliant cultivar acquired by the MS Foundation for Research and Dissemination of Agricultural Technologies (Fundação MS Para Pesquisa e Difusão de Tecnologias Agropecuárias - FUNDAÇÃO MS). Plants emerged on March 15th, 2011. The final average stand of plants was 1,220 plants per hectare. The plants reached physiological maturity on June 11th, 2011 at 88 DAE, and the harvest was performed on June 21st, 2011.

The following dependent variables were evaluated: (a) root and shoot dray mass in three different seasons (35 DAE when the plants were at the beginning of flowering; 45 DAE at which the plants were in the full flowering stage and beginning of the filling phase; and 55 DAE when the plants were in the filling phase); (b) grain yield; and (c) fixed oil content of the grain.

For evaluation of the dry root and shoot masses of crambe, three plants located along the boundary of each plot were harvested using a hoe, and all roots and shoots of the plant were removed as completely as possible at all collection times. Samples were collected at 35, 45 and 55 DAE. Roots were separated from the shoots in the field, and both were placed in paper bags and identified according to the plot. The material was taken to the laboratory where it was washed with distilled water, and the material was then placed in a forced air drying oven at 65°C for 72 h to be weighed.

To determine the grain yield (kg ha^{-1}), the lateral rows were discarded along with 0.5 m from each side of the plot. Three linear meters of the three central lines (totaling 4.05 m^2) from the center of each plot were harvested. Harvest was performed manually, and the grains were placed in paper bags and transported to the laboratory, where they were cleaned and dried in a forced air circulation oven at 65°C for 72 h to standardize the moisture content before weighing.

For determining the oil content in crambe, the grains were crushed manually in a porcelain crucible. The sample (5 g) was

Table 1. Effect of increased soil base saturation (V%) and application of different N doses (kg ha^{-1}) on the crambe crop in field conditions (Table of mean squares).

Unfoldings	Root dry mass (g $plant^{-1}$)			Shoot dry mass (g $plant^{-1}$)			Yield	Oil content
	35 DAE	45 DAE	55 DAE	35 DAE	45 DAE	55 DAE	Kg ha^{-1}	(%)
N	0.00264*	0.1568**	0.02030 ns	0.55648**	0.434 ns	5.1447ns	48203.564**	0.42303 ns
Regression (V%)	Linear **	Linear**	Linear*	Linear**	Linear*	ns	Quadratic*	Linear**
Interaction (V%xN)	0.00080ns	0.00474**	0.01323 ns	0.01338 ns	0.945**	9.177**	8455.921**	0.33412 ns
CV (%)	29.56	19.08	35.46	35.57	25.56	30.33	9.65	1.83

DAE: Days after emergence; ns: non-significant; *: Significant (P ≤ 0.05); **: Significant (P ≤ 0.01).

Table 2. Root dry mass of crambe (g $plant^{-1}$) as a function of the addition of N doses and harvest at different times (Averages of 16 observations).

Nitrogen (kg ha^{-1})	Harvest time (DAE)		
	35	45	55
Control	0.083[b]	0.156[b]	0.257[a]
40	0.090[ab]	0.194[a]	0.327[a]
80	0.107[a]	0.218[a]	0.302[a]
CV (%)	29.56	19.08	35.46

Averages followed by the same letter at the same harvest time do not differ according to Tukey's test (p ≤ 0.05).

added to the cellulose thimble of the fat analyzer (model TE-044-8/50; TECNAL) for eight simultaneous tests, and hexane was used as the solvent (200 ml per sample). The temperature was controlled electronically at 120°C, and extraction took place for 4 h.

In the statistical analysis and for the levels of base saturation, a polynomial regression analysis was performed. For the three N doses, an analysis of variance was performed, and Tukey's test (5%) was performed when necessary. The statistical program used was Assist at 7.6 Beta.

RESULTS AND DISCUSSION

The effect of the treatments on root dry mass development of crambe depended on the assessment period. At the first time of plant collection (35 DAE), the statistical analysis showed a significant effect for both N and increased soil base saturation, but there was no significant interaction effect (V% × N) (Table 1). At the second time of plant collection (45 DAE), a significant interaction effect (V% × N) was observed. Moreover, at the third time of plant collection (55 DAE), a significant effect was only observed for the analysis of variance for regression of soil base saturation levels (Table 1).

At the two times (35 and 45 DAE) when a significant effect was observed, the highest N dose used (80 kg ha^{-1}) did not differ from the N dose of 40 kg ha^{-1} although it differed from the control (Table 2), thereby indicating that crambe did not respond to higher N doses for this parameter evaluated and at the conditions of this study.

In evaluating the effect of increased base saturation for the harvests at 35 and 55 DAE where the interaction (V% × N) was not significant (Table 1), a linear effect was observed for root dry mass until a base saturation of 60% (Figure 3).

In the collection of plants performed at 45 (DAE), when the interaction (V% × N) was significant (Table 1), unfolding was performed for the effect of increasing saturation in each N fertilization, and the observed behavior depended on the N dose applied. In the control (no N application), the behavior was polynomial (quadratic), and the maximum point of the regression equation for base saturation was 47.12%. In plots that received the 40 kg ha^{-1} N dose, the behavior was linear, and in plots that received the 80 kg ha^{-1} N dose, the behavior was not significant (Figure 4).

Comparison of the effect of N doses on development of the crambe root system with literature is difficult due to the low number of published papers. In a study of crambe in a nutrient solution, Brito (2009) did not report a significant result for N doses on the development of the crambe root system. Alves et al. (2010) studied greenhouse conditions and found a linear effect for N application of up to 160 kg ha^{-1} using urea as a N source.

In evaluating the effect of increasing soil base saturation, the regression analysis showed a linear effect for the three root sampling times (35, 45 and 55 DAE) indicating that this evaluated variable for crambe was strongly influenced by soil base saturation (Table 1). These results were not in agreement with those obtained in literature where Carvalho et al. (2012) found a quadratic

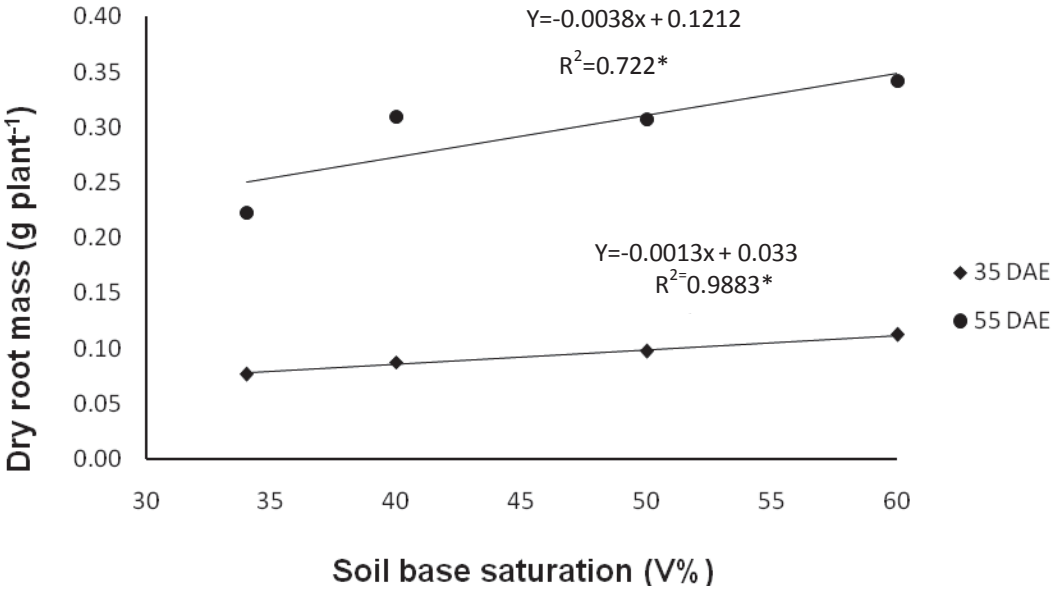

Figure 3. Root dry mass of crambe (g plant^{-1}) as a function of increasing soil base saturation (V%) harvested at 35 and 55 DAE. Averages of 12 observations.

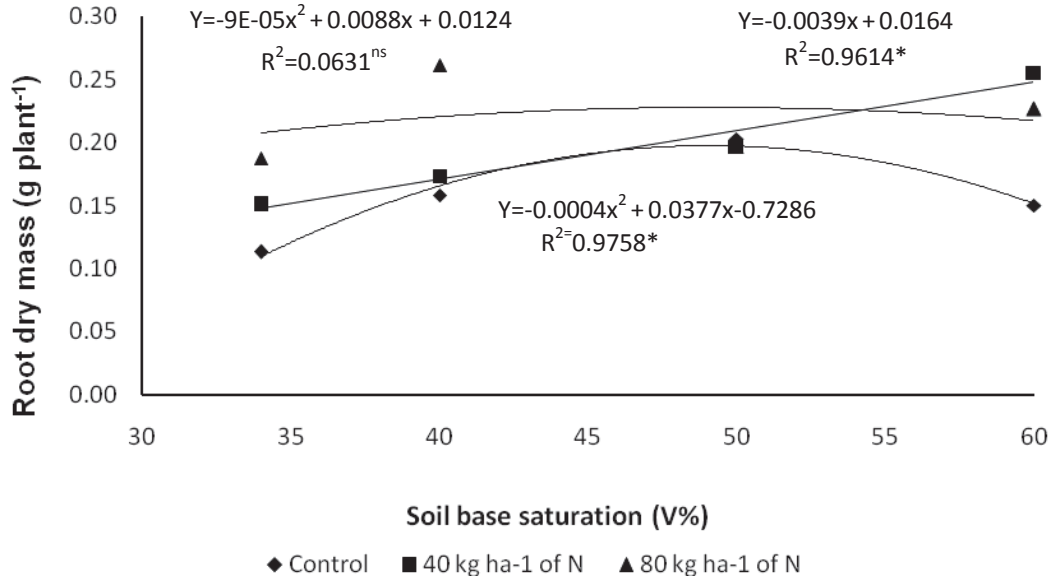

Figure 4. Root dry mass of crambe (g plant^{-1}) as a function of the increased soil base saturation (V%) harvested 45 days after emergence (DAE). Averages of 12 observations. ns = non-significant.

response as a function of the soil base saturation level for dry root weight in a pot experiment. These authors reported that the best base saturation level was 45% and that the dry root weight decreased at the 60 and 75% levels, which was different from that found in the present study. Janegitz et al. (2010) increased the soil base saturation up to 80% and obtained no effect on the development of the crambe root system. These discordant results found in literature that were produced in soil were conducted in a similar Latosol to that used in this study. Therefore, further studies are needed to establish an efficient implementation of this culture to provide better conditions for root development and, consequently, the plant as a whole.

In the statistical analysis to evaluate the effect of the treatments on dry shoot mass development, a significant

Table 3. Dry shoot mass of crambe (g plant^{-1}) as a function of N doses and different harvest times (Averages of four observations).

Base saturation (V%)	Harvest times (DAE)								
	35			45			55		
	Nitrogen doses (kg ha^{-1})								
	Cont	40	80	Cont	40	80	Cont	40	80
34	0.37^{b}	0.50^{ab}	0.82^{a}	1.22^{a}	1.53^{a}	1.81^{a}	4.57^{a}	1.85^{b}	4.38^{a}
40	0.43^{b}	0.60^{ab}	0.88^{a}	2.36^{a}	1.76^{a}	2.22^{a}	3.87^{a}	4.91^{a}	4.27^{a}
50	0.54^{a}	0.75^{a}	0.85^{a}	2.15^{a}	2.22^{a}	1.82^{a}	4.09^{b}	6.70^{a}	3.53^{b}
60	0.71^{a}	0.78^{a}	0.99^{a}	1.37^{b}	2.90^{a}	2.00^{b}	3.13^{b}	6.21^{a}	3.63^{b}
CV(%)		35.57			25.56			30.33	

Averages followed by the same letter on the line for the same harvest period do not differ according to Tukey's test (P≤0.05). Cont=control.

interaction (N × V%) was observed for the second and third sampling times (45 and 55 DAE) (Table 1). In the evaluation at 35 DAE and base saturation levels of 34 and 40 (V%), the addition of N differed from the control, and the highest N dose employed (80 kg ha^{-1}) did not differ from the 40 kg ha^{-1} N dose (Table 3). In the second evaluation period (45 DAE), the effect of N addition was only observed for the highest base saturation level used (V% = 60) in which the 40 kg ha^{-1} N dose differed from both the control and the 80 kg ha^{-1} N dose (Table 3). In the third evaluation period (55 DAE), the effect of N doses also depended on the saturation level observed. For the first saturation level (V% = 34), the 80 kg ha^{-1} N dose and the control differed from the 40 kg ha^{-1} N dose. At the highest base saturation levels (V% = 50 and 60), the 40 kg ha^{-1} N dose differed from the control and the highest N dose (80 kg ha^{-1}).

Camargo et al. (2010) also obtained a significant response on the development of dry shoot mass of crambe 60 days after planting when mineral nutrients (NPK) were applied during planting and N was applied in coverage. In an experiment using a nutrient solution, Brito (2009) obtained a significant response to N (nitrate) for leaf dry mass and a non-significant response for the stem dry mass. It is expected that N has an effect on the development of crambe shoots. According to Oliveira et al. (1996), when N is deficient, plants are stunted, and the stem and branch are slender in addition to the leaves having a color between pale green and yellow. The same author reported that N fertilization well applied in coverage has the ability to meet all of the needs of the culture and, thus, increases its productivity. This significant effect of N on shoot growth of other plants is known. Wright et al. (1988) showed that N treatment of rapeseed prolongs the life of leaves, improves flowering and increases the general uptake of crops.

The highest mean grain yield obtained in this study was 582 kg ha^{-1}. This yield may be considered low compared to that found in literature because Pitol et al. (2010) reported an average yield of 1,000 to 1,500 kg ha^{-1} for the FMS Brilliant cultivar. A literature review suggested that several authors obtained grain yields for crambe higher than 1,500 kg ha^{-1} in soil conditions similar to those used in the present study (Red Latosol) (Jasper et al., 2010b; Santos et al., 2012; Rogério et al., 2012, 2013). Figure 1 shows that precipitation is one of the factors that may have negatively influenced grain yield. In soils with good capacity to retain water, Roscoe et al. (2010) reported that crambe produces satisfactorily when it receives at least 50 mm of water distributed in two rainfalls after planting. These authors also reported that the ideal amount of water varies between 150 and 200 mm, particularly before full flowering, and they also suggested rainfall after full flowering is not necessary. According to data collected at the meteorological station of the University of Rio Verde (FESURV), more than 400 mm of precipitation was recorded during the study period, which is equivalent to twice the minimum required for this culture, as described by Roscoe et al. (2010). Nevertheless, distribution of rainfall was irregular and was concentrated in the vegetative growth phase (March) with small amounts in the later stages. Considering that the early process of crambe graining began at the end of April in this study, the amount of precipitation in the period of full flowering and grain filling stage may have impaired the productivity of this crop. Thus, the data reported here was in disagreement with that reported by Roscoe et al. (2010). To ensure higher yields, more information is needed regarding the behavior of this crop under different rainfall intensities to more clearly define the water requirements of the crop. In addition, no significant attack of pests and diseases that could have negatively influenced grain yield was observed, and plants apparently developed normally.

For grain yield, the statistical analysis showed a significant effect for the V% x N interaction (Table 1). At the lowest base saturation level (V% = 34), which was the natural soil base saturation, the addition of 40 kg ha^{-1} N increased productivity by 38.2%, thereby significantly differing from the control. However, use of 80 kg ha^{-1} N did not statistically differ from the lowest N dose, thereby indicating that high N doses should not be used in soils

Table 4. Grain yield for crambe (kgha^{-1}) and yield relative to control (in parentheses) as a function of the increased soil base saturation (V%) and N doses (Averages of four observations, CV(%)=9.65).

Base saturation	Nitrogendoses(kgha^{-1})		
(V%)	Control	40	80
34	372(100)^{b}	514(138)^{a}	494(133)^{a}
40	410(110)^{b}	445(120)^{ab}	509(137)^{a}
50	473(127)^{b}	582(156)^{a}	503(135)^{b}
60	385(103)^{b}	534(143)^{a}	398(107)^{b}

Averages followed by the same letter on the line do not differ according to Tukey's test (P≤0.05).

that are not corrected because the crambe plant is not able to respond to the treatment (Table 4).

When the soil base saturation was increased to 40%, the average of the control yield (410 kg ha^{-1}) did not differ from the first N dose (40 kg ha^{-1}) in which a yield of 445 kg ha^{-1} was observed, thereby indicating that this increased base saturation was equivalent to the application of N (Table 4). In the higher levels of soil base saturation (50 and 60%), the yield observed for 40 kg ha^{-1} N was significantly higher than that of the control, but it was also significantly lower than that with 80 kg ha^{-1} N (Table 4), thereby indicating that higher doses of N did not increase crambe productivity but may even reduce it. The highest average grain yield obtained in this study was 582 kg ha^{-1}, and it was obtained when plants were subjected to a soil base saturation of 50% and a N dose of 40 kg ha^{-1}. This productivity was 56.4% higher than in the control (Table 4).

Broch and Roscoe (2010) also found a response to N fertilization in the crambe culture in soils with low OM. However, the results found by these authors showed a beneficial effect with quadratic behavior for N addition and decreased productivity for N dosages greater than 35 kg ha^{-1}. The data obtained in the present study confirmed the results obtained by Broch and Roscoe (2010) regarding N doses. In this study, the use of 80 kg ha^{-1} N resulted in lower average grain yields compared to 40 kg ha^{-1} N for all base saturations evaluated (Table 4).

Different from that obtained in this study, Freitas (2010) obtained no significant response of crambe grain yield when 60 and 120 kg ha^{-1} N were used in an experiment performed during two consecutive years. However, this author used urea as a N source and reported that the fertilizer was spread manually in the plant rows, which was a small amount when considering the amount of rainfall that occurred during the experiment. In addition, soil moisture content may have been low, which may have increased N losses by volatilization. Lara Cabezas et al. (1997) reported that when selecting urea as the N fertilizer and applying it via spreading without incorporation into the soil, N losses by the ammonia volatilization process in this system can reach 78% of the fertilizer applied. Lunelli (2012) found no significant effect in the FMS Brilhante crambe cultivar cultivated in a Red Latosol when 90 kg ha^{-1} N in the form of urea was applied to the soil, and this lack of significant effect may have occurred because the soil was rich in nutrient content and OM, in contrast to the conditions under which the present study was conducted. Another possibility is that the N dosage used by these authors (90 kg ha^{-1}) is a high dose for this crop because the results obtained in the present work and those obtained by Broch and Roscoe (2010) indicated that doses greater than 40 kg ha^{-1} N can decrease grain production of this crop. In a study using a nutrient solution, Brito (2009) also obtained no significant effect of N doses (NO_3^-) on grain yield.

In evaluating the effect of base saturation for each N dose on crambe productivity, the analysis of variance for regression showed a significant effect only for the linear model of 40 and 80 kg ha^{-1} N, but the coefficient of determination (R^2) was low, thereby indicating that although significant, the model did not satisfactorily explain the results obtained in this work. Working in greenhouse conditions, Janegitz et al. (2010) also obtained no significant response for increased base saturation to 80% for the crambe crop. This non-significant result on yield was not expected because Broch and Roscoe (2010) reported that crambe is a plant sensitive to soil acidity and that its productivity is severely impaired when in the presence of exchangeable Al and low levels of Ca and Mg. In the present study, the sum of Ca and Mg levels in the soil was equal to 2.3 cmol$_c$ dm^{-3}. Therefore, the presence of Ca, presence of Mg, low yields and the absence of toxic Al may have contributed to the base saturation levels not having a significant effect.

In evaluating the effect of the treatments on oil content of crambe, the statistical analysis showed no significant effect for N and for the interaction (V% x N) (Table 1), but the analysis of variance for regression was highly significant for the linear model of soil saturation (Figure 5). These results were in agreement with those obtained in the literature for other oil crops. Smiderle and Costa (2010) worked with soil base saturations ranging from 30 to 75%, and they obtained a linear increase in the oil content of sunflower.

Other authors have also reported finding no effect of N on the oil content of oilseeds. When working with an

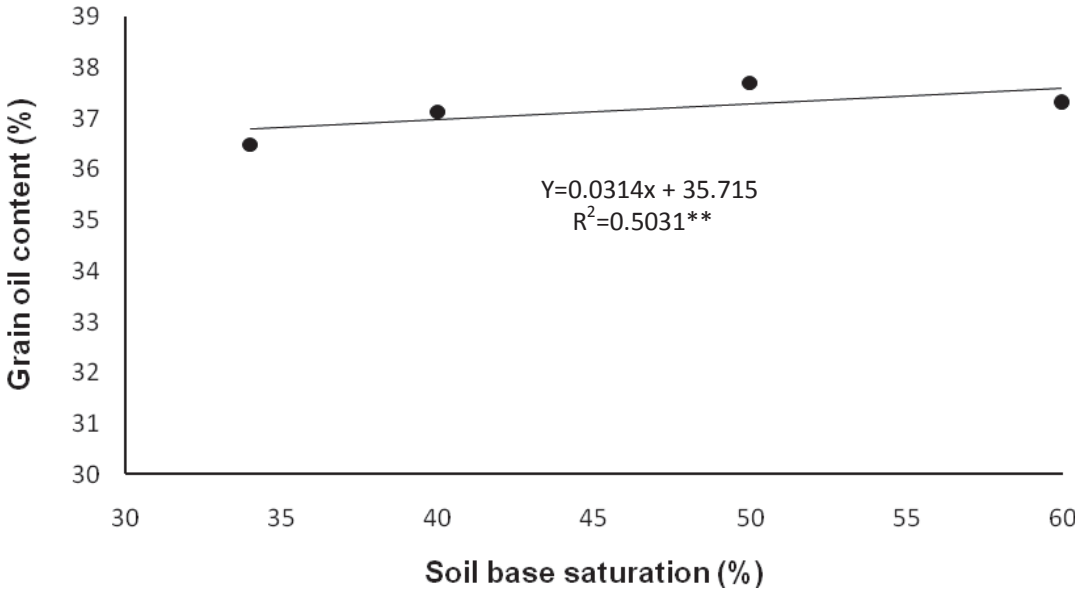

Figure 5. Oil content of crambe grains (%) as a function of the increased soil base saturation (V%). Averages of 12 repetitions. CV(%) = 1.83.

oilseed crop from the same family as crambe, namely rapeseed (*Brassica napus*), Dreccer et al. (2000) reported that no significant effect of N on oil content is observed. A review by Rathke et al. (2006) reported that most results show that N application often results in an increase in productivity and protein content as well as a decrease in oil content for the rapeseed culture. These authors report in their work an inverse correlation for the rapeseed culture, particularly between protein and oil content, and Brito (2009) confirmed these results in the crambe culture. In one experiment using a nutrient solution, this author observed that the addition of higher doses of nitrate reduces oil content in relation to that observed in the control. Thus, when an oilseed crop is fertilized with high N concentrations, contents of this nutrient are increased in the tissues, reducing the synthesis of oils and favoring the metabolic pathway of protein accumulation in the achenes (Castro et al., 1999).

Conclusions

1. The addition of N doses influenced root development, shoot development and productivity of crambe. The optimal dosage was 40 kg ha^{-1} N;
2. Base saturation influenced root development, shoot development, productivity and oil content of crambe, and the optimal base saturation level was 50%.

Conflict of Interest

The authors have not declared any conflict of interest.

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Full Length Research Paper

Estimates of genetic components for yield and yield related traits of Tannia (*Xanthosoma sagittifolium* (L.) Schott) genotypes at Jimma, Southwest Ethiopia

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Tannia is one of the most important root and tuber crops for food, feed and industrial applications worldwide. However, the progress to variety development in Ethiopia is so slow due to lack of adequate germplasm characterization and agronomic evaluation for yield and quality. Therefore, a total of 64 tannia genotypes were studied to determine the extent of genetic variability among genotypes at Jimma agriculture research center during 2013/2014 cropping season. 62 of the genotypes were collected from south, south western and western parts of Ethiopia and the rest two were introduced from Cuba, laid out in 8 × 8 simple lattice design. Analysis of variance revealed significant (P≤0.01) differences for most of the characters, indicating the existence of variability among the studied genotypes. High phenotypic coefficient of variation (PCV) along with moderate to high genotypic coefficient of variation (GCV) as well as high heritability coupled with high genetic advance as percent of the mean were obtained for number of suckers per plant, number of cormel per plant, total yield per plant, corm and cormel fresh weight per plant. This indicates that there is an opportunity and potential for further utilization of its genetic improvement through selection and hybridization. However, the presence of morphological variation between genotypes is not a guarantee for high genetic variation. Hence, there is a need to confirm genotype-environment interactions and use biotechnological approaches as a complementary to this study.

Key words: Genetic advance, genetic variability, genotypic coefficient of variation, heritability.

INTRODUCTION

Tannia is an herbaceous, monocotyledonous, perennial stem tuber crop that is widely cultivated in tropical and subtropical regions of the world. Tannia belongs to the family Araceae and originally came from tropical America (Ramesh et al., 2007). Tannia ranked sixth in planted area and production after cassava, potato, sweet potato, yam and taro in the world (Perez, 2010).

Root and tuber crops including tannia can play multi-purpose roles in the global food system to address the ever increasing demand for food and feed millions of

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people (Ceballos, 2009; Lebot, 2009; Ndabikunze et al., 2011). According to FAOSTAT (2012), world production for taro and tannia in 2011 was 10.37 million tonnes. Africa as a continent produces more than 71% of world production. Globally, tannia is an important food for about 400 million people (Lebot, 2009) and in Ethiopia a total of 1.5 million farmers mainly in Southern Nations Nationalities and Peoples (SNNP) region (0.96 million) and in Oromia region (0.5 million) are dependent on taro and tannia as their food source (CSA, 2012b). During 2011/2012 production year, taro and tannia production area in Ethiopia reached 39,696 hectares (CSA, 2012b) with total production of 315,242 tonnes of which 81.2% is used for human consumption and 11.5% reserved for planting material (CSA, 2012a). Even though tannia has many roles, it is unexploited and neglected crop. Lack of improved varieties and need of large planting material (Onokpise et al., 1999), rare natural flowering and seed setting (Mbouobda et al., 2007), transmission of pathogens specially dasheen mosaic virus (DsMV) via vegetative propagation which can cause yield losses up to 90% (Onokpise et al., 1999; Reyes et al., 2006; Mbouobda et al., 2007) are major constraints of tannia production.

To overcome these constraints and improve production and productiveness of tannia, research has been conducted in different countries. In Costa Rica, genetic variability is generated by induction mutation (Saborio et al., 2004), in Ghana, Blay et al. (2004) irradiated tannia shoot tips with gamma rays to generate variability, In Bangladesh, protocol establishment for micro propagation and vitro callus regeneration were performed (Paul and Bari, 2007). Also, Onokpise et al. (1992) sprayed Gibberellic acid (GA_3) to induce flowering and seed setting.

But tannia in Ethiopia is still neglected, so far no improved varieties are available and no characterization and other works have been undertaken. Even though, the national average yield level of tannia in Ethiopia is greater than the global average yield of 7.4 tonnes/ hectar (FAOSTAT, 2012), its productivity is far below the crop's potential which is 30 to 60 tonnes/hectare (Lebot, 2009; Mwenye et al., 2010). For crop improvement program genetic variability is an essential prerequisite for obtaining high yielding, quality, pest and disease resistant varieties (Paul and Bari, 2012).

As Amsalu and Tesfaye (2006) and Tewodros (2008) state, tannia has a large gene pool in south and southwest Ethiopia in farmers' field and homesteads. Recently some germplasm collection and conservation works have been started by agricultural research centers (Amsalu et al., 2008). Nevertheless, the collected genotypes have never been characterized or evaluated for desirable characteristics. So, there is paucity of information in respect to their genetic variability and agronomic performance. Therefore, the present study was conducted with the objective of determining the extent of genetic variability based on quantitative characters.

MATERIALS AND METHODS

Study area

The experiment was conducted at Jimma Agricultural Research Center (JARC) located at 366 km south west of Addis Ababa. The site is situated at a latitude 7° 46' N and longitude 36° E with an altitude of 1753 m.a.s.l. The soil of the study area is Eutric Nitisol with a pH of 5.3. The area receives mean annual rainfall of 1432 mm with maximum and minimum temperature of 29.2 and of 8.9°C, respectively.

Expermental design

A total of 64 tannia genotypes having same cormel size, 62 genotypes collected from south, south western and western parts of Ethiopia and two introductions from Cuba (Table 1), laid out in 8 × 8 simple lattice design using single row plots of 8.25 meter long, each spaced 1 m apart between rows and 0.75 m between plants. There were 11 plants row^{-1} and the middle five plants were randomly selected and used for data collection. After the crop established well, earthing up and weeding were carried out when necessary.

Data collection

Descriptor of tannia developed by International board for Plant Genetic Resources (IBPGR, 1989) was followed for data collection. 16 quantitative data were used, most of which were distinguished as highly heritable traits. Measurements of above ground morphological characters were carried out from those selected middle five plants in each plot at 5^{th} to 6^{th} months after planting when the plants have reached their peak above ground vegetative growth, while subterranean traits were evaluated at harvest (nine and half months after planting). Data were collected on traits: Lamina length (cm), lamina width (cm), number of suckers per plant, petiole length (cm), plant height (cm), plant canopy diameter(cm), corm length(cm), corm diameter(cm), cormel length (cm), cormel diameter (cm), corm fresh weight per plant (kg), cormel fresh weight per plant (kg), total root yield per plant (Kg), number of cormels per plant, corm dry matter content (%) and cormel dry matter (%).

Statistical analysis

Collected data were subjected to ANOVA based on simple lattice design using SAS version 9.2 (SAS, 2008). Then the differences between genotypes mean were compared using LSD (Least significance difference) at 5% probability level. The ANOVA model for simple lattice design is:

$$Y_{ijklm} = \mu + t_i + \beta + \chi_{(k)} + y_l + \pi_m + o_{ijklm}$$

Where: Y_{ijklm} = response of Y trait from the i^{th} genotypes, j^{th} replication, μ = Overall mean effects, t_i = Effects of i^{th} level of treatments, β = Effects of j^{th} level of replication, χ_k = Effects of K^{th} level of blocks within replications (adjusted for treatments), y_l = Effects of l^{th} level of intra block error, π_m = Effects of the m^{th} randomized complete block error and O_{ijklm} = is a random error component.

Table 1. List of genotypes of tannia studied studied at Jimma, 2013/2014.

Genotype	Destrict	Kebele/Village	Altitude (masl)	Genotype	Destrict	Kebele/Village	Altitude (masl)
AAGT003	Chena	Bobakrcha	2100	AAGT109	Gesha	Hinigdo	1640
AAGT008	Bench	Kochi	1380	AAGT112	Gimbo	Kaikelo	1600
AAGT020	Bench	Wachamaji		AAGT116	Gimbo	Kembo	1820
AAGT022	Bench	Aman Gonji	1380	AAGT120	Chena	Kutasheorai	1820
AAGT030	Bench	Mizan		AAGT121	Chena	Agaro	1980
AAGT031	Bench	Koda	2040	AAGT127	Chena	Culish	
AAGT034	Chena	Ralakocho Bacha	1960	AAGT132	Bench	Aman	
AAGT035	Decha	Chalta	1620	AAGT135	Bench	Gerika	1460
AAGT036	Decha	Shapa	1840	AAGT138	Sheka	Bukita	1460
AAGT043	Decha	Deha	1880	AAGT144	Sheka	Selale	1640
AAGT045	Decha	Chiri		AAGT148	Sheka	Wesheka	1660
AAGT046	Decha	Chiri		AAGT152	Sheka	Shimi	1320
AAGT051	Gimbo	Kaiketa	1860	AAGT155	Sheka	Gizm	
AAGT052	Gimbo	Beyamo	1680	AAGT159	Yeki	Korech	1140
AAGT054	Gimbo	Aman	1700	AAGT163	Yeki	Korech	1380
AAGT058	Gimbo	Getoacho	1640	AAGT171	Mesha	Tugri	1840
AAGT061	Gimbo	Shamba	1500	AAGT176	Mesha	Toba	2220
AAGT065	Decha	Erma	1860	AAGT177	Mesha	Keja	2140
AAGT069	Decha	Adaiminja	1860	AAGT178	Mesha	Chewaka	1840
AAGT077	Decha	Muga	1900	AAGT180	Gesha	Asho	2160
AAGT080	Decha	Gedam	1680	AAGT183	Gesha	Yershiniti	2180
AAGT083	Telo	Tura	2020	AAGT186	Mesha	Gecha	
AAGT085	Telo	Shadie	1640	AAGT188	Yeki	Chati	1820
AAGT088	Telo	Felegeselam	2060	AAGT193	Yeki	Gendekore	1260
AAGT092	Gimbo	Beymo	1660	AAGT195	Yeki	Sbosha	1220
AAGT093	Gimbo	Kicho	1720	AAGT199	Yeki	Bechi	1180
AAGT094	Gimbo	Kuti	1760	AAGT202	Yeki	Kura Alamo	1220
AAGT097	Gimbo	Emicho	1820	AAGT205	Yeki	Alamo	1380
AAGT099	Gimbo	Saja	2060	AAGT208	Chena	Tofa	1820
AAGT100	Gimbo	Medaobo	1600	0002/07			
AAGT102	Gimbo	Medaobo	1560	0003/07			
AAGT106	Gimbo	Konda		AAGT174	Mesha	Gtimo	2250

Genotypic (σ^2g), environmental (σ^2e) and phenotypic (σ^2p) variance component were computed as (Singh, 2001) as follow:

$$\sigma^2 g = \frac{\text{MSg - MSe}}{r}, \quad \sigma^2 p = \sigma^2 g + \sigma^2 e \text{ and } \sigma^2 e = MS$$

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

Phenotypic coefficient of variation (PCV) and genotypic coefficient of variation (GCV) were estimated according to the method suggested by Burton and De Vane (1953), as:

$$\text{PCV} = \frac{\sqrt{\sigma^2 p}}{\bar{x}} \times 100$$

$$\text{GCV} = \frac{\sqrt{\sigma^2 g}}{\bar{x}} \times 100$$

Where: is the grand mean value of the trait.

Heritability in Broad Sense (h^2B) estimated as described by Allard (1960) as follow:

$$\text{h}^2\text{B} = \frac{\sigma^2 g}{\sigma^2 p} \times 10($$

Expected Genetic Advance (GA) and Expected genetic advance as percent of the mean (GAM) were predicted as suggested by Johnson et al. (1955):

$$GA = (K).(\sigma p).(h^2 B)$$

$$\text{GAM} = \frac{GA}{\bar{x}} \times 100$$

Where: K = selection differential which varied with selection intensity (5% intensity was used at which K = 2.06), σp = phenotypic standard deviation, h2B = heritability and $\bar{x}$ = population mean.

Table 2. Mean squares of tannia genotypes for 16 quantitative characters studied at Jimma, 2013/14.

Character	Mean square		CV (%)	R^2
	Treatment	Error		
SU	0.76**	0.15	16.73	88.4
LL	6.32**	1.4	4.87	91.89
LW	6.18**	1.5	5.42	89.11
PLC	61.01**	14.04	5.69	87.75
PTLg	35.87**	8.88	6.54	89.61
Ph	57.96**	15.91	7.08	88.78
COL	2.48*	1.43	11.52	77.89
CLD	0.44**	0.17	7.9	80.41
NCL	14.48**	4.9	18.84	83.98
CML	0.95** [ad.]	0.48	8.04	81.79
CMD	0.93*	0.59	9.27	75.5
COW	0.0226**	0.008	19.7	84.5
CMW	0.0064**	0.0014	16.09	91.62
CMDM	10.41**	4.09	7.24	81.78
CODM	11.23**	4.46	7.65	78.7
Tot Yi.	0.043**	0.0137	17.01	87.55

**, * significance at 0.01 and at 0.05 probability level; [ad] = adjusted treatment mean. SU = number of suckers per plant, LL = lamina length (cm), LW = lamina width (cm), PLC = plant canopy diameter(cm), PTLg = petiole length (cm), Ph = plant height (cm), COL = cormel length (cm), CLD = cormel diameter (cm), NCL = number of cormels per plant, CML = corm length (cm), CMD = corm diameter (cm), COW = cormel fresh weight per plant (kg), CMW = corm fresh weight per plant (kg), CMDM = corm dry matter content (%), CODM = cormel dry matter content (%), TotYi = total root yield per plant (kg).

Where: K = selection differential which varied with selection intensity (5% intensity was used at which K = 2.06), σp = phenotypic standard deviation, h^2B = heritability and = population mean.

RESULTS AND DISCUSSION

The analysis of variance showed presence of significant variations (P ≤ 0.05) among genotypes for the studied characters (Table 2). This significant variation among tested genotypes for the characters is the result of combinations of genotypic and phenotypic effect (Acquaah, 2012). Such a significant variation was reported previously such as: Paul and Bari (2012) were reported a significant difference among tannia genotypes for characters like plant height, petiole length, cormel breadth, corm breadth, cormel weight, corm weight and yield per plant. Similarly, Tewodros (2013) reported that petiole length and plant canopy diameter showed highly significant variation; but he reported that non-significant variation of lamina length, lamina width, number of sucker per plant, plant height and tuber fresh weight among taro genotypes in Ethiopia.

The range and mean of genotypes for the studied characters also showed wide ranges of variation (Table 3) like number of sucker per plant (1.19 to 4.42), lamina length (20.81 to 29.61 cm), lamina width (18.44 to 27.94 cm), plant canopy diameter (56.33 to 82.03 cm), petiole length (37.72 to 57.52 cm), plant height (44.68 to 72.72 cm), cormel length (7.83 to 13.13), number of cormels per plant (7.38 to 13.57), cormel weight (0.27 to 0.74 kg/plant), corm weight (0.13 to 0.43 kg/plant), corm dry matter content (21.5 to 32.5%), cormel dry matter content (20 to 32.5%) and total yield per plant (0.44 to 1.10 kg/plant). Moreover, the difference between the minimum and the maximum mean values were high, indicating the availability of variation for improvement through selection. Such a wide range of variation in plant height, plant canopy diameter, lamina width and lamina length gives good opportunity for selection to have desired plant characters by selection or hybridization with respect to spacing (plant population per hectare), leaf area with respect to other physiological characters like transpiration and photosynthesis (light harvesting structure) and availability of moisture to improve productivity per hectare base.

Based on the mean values, the average value was almost twice that of the minimum mean values for character cormel fresh weight and corm weight indicating that their maximum contribution to the total variability observed among the genotypes was high. More than half of the tested genotypes had mean corm and cormel dry

Table 3. The range and the mean values of Tannia genotypes for 16 characters studied at Jimma, 2013/14.

Character	Maximum mean		Minimum mean		Grand mean
	Value	Acc.	Value	Acc.	
SU	4.42	AAGT102	1.19	AAGT127	2.36
LL	29.61	AAGT152	20.81	AAGT199	24.29
LW	27.94	AAGT152	18.44	AAGT199	22.65
PLC	82.03	AAGT102	56.33	AAGT135	65.51
PTLg	57.52	AAGT069	37.72	AAGT199	45.59
Ph	72.72	0003/07	44.68	AAGT171	56.33
COL	13.13	AAGT152	7.83	AAGT003	10.38
CLD	5.98	AAGT163	3.33	AAGT159	5.19
NCL	13.57	AAGT106	7.38	AAGT003	11.67
CML	9.64	0003/07	6.43	AAGT171	7.94
CMD	10.15	AAGT094	6.59	AAGT159	8.28
COW	0.74	AAGT183, 0003/07	0.27	AAGT171	0.454
CMW	0.43	AAGT069	0.13	AAGT199, AAGT176, AAGT109	0.24
CMDM	32.50	AAGT132	21.50	0002/07	27.94
CODM	32.50	AAGT202	20.00	0002/07	27.61
Tot. Yi	1.10	0003/07	0.44	AAGT127, AAGT159	0.69

SU = number of suckers per plant, LL = lamina length (cm), LW = lamina width (cm), PLC = plant canopy diameter(cm), PTLg = petiole length (cm), Ph = plant height (cm), COL = cormel length (cm), CLD = cormel diameter (cm), NCL = number of cormels per plant, CML = corm length (cm), CMD = corm diameter (cm), COW = cormel fresh weight per plant (kg), CMW = corm fresh weight per plant (kg), CMDM = corm dry matter content (%), CODM = cormel dry matter content (%), TotYi = total root yield per plant (kg).

matter content of above the overall mean of the genotypes (27.94 and 27.61% respectively). Similarly, 25, 42 and 36% of the genotypes showed higher weight of cormel, corm and total yield than the grand mean yield 0.454, 0.24, 0.69 kg/plant respectively. Hence, there is an opportunity to find genotypes among the tested entries that give better yield of cormel and corm as well as genotypes which have higher dry matter content of corm and cormel. Such a wide variation were observed before and the results were in agreement with the findings of Opoku-Agyeman et al. (2004) who reported a wide ranges of variation of 78 tannia genotypes for characters like petiole length, plant height, number of cormel per plant and cormel fresh weight in Ghana. Similarly, ReyesCastro et al. (2005) reported variation between tannia genotypes for traits of plant height, number of suckers, number of cormels per plant, cormel length, cormel weight and total yield.

The maximum phenotypic variances were obtained for plant canopy (37.52) followed by plant height (36.94) and petiole length (22.37). As in Table 4 the genotypic variances for these characters were also high for plant canopy (23.49) followed by plant height (21.03) and petiole length (13.5), indicating that the genotype could be reflected by the phenotype and the effectiveness of selection based on phenotypic performances of these characters. Relatively lower variances were observed for number of suckers per plant, cormel diameter, cormel length, corm diameter, corm length, corm fresh weight, cormel fresh weight and total root yield per plant.

On the other hand, PCV ranged from 8.09 for lamina length to 28.64 for number of sucker per plant and GCV ranged from 4.98 for cormel diameter to 23.48 for number of sucker per plant. According to Deshmukh et al. (1986) PCV and GCV values of more than 20% are considered as high, values less than 10% as low and values between 10 and 20 as moderate. Hence, number of sucker per plant (GCV = 23.48; PCV = 28.64), number of cormels per plant (GCV = 18.64; PCV = 26.49), cormel fresh weight (GCV = 18.81; PCV = 27.24), corm fresh weight (GCV = 21.13; PCV = 26.42) and total yield per plant (GCV = 17.54; PCV = 24.40) showed moderate to high GCV along with higher PCV. While the rest showed lower PCV and GCV.

In the present study, almost all characters exhibited higher PCV than their corresponding GCV (Table 4), indicating the apparent variations in the genotypes were not only due to genotypic effect but also due to environmental influences, since phenotypic variances were contributed by the effect of interaction of genotypes and environment (Acquaah, 2012). However, the difference between PCV and GCV were relatively narrow, shows that the observed variations for the trait were mostly due to genetic factors so, selection could be advanced here. The PCV and GCV gap between number of cormel per plant, corm diameter and cormel length was wider indicating the greater contribution of environment on these traits. This is in support of Bisne et al. (2009) who stated that high phenotypic variations composed of high genotypic variations and less of environmental

Table 4. Estimates of phenotypic (σ^2p), environmental (σ^2e) and genotypic (σ^2g) variances, phenotypic coefficient of variation (PCV) and genotypic coefficient of variation (GCV), heritability in broad sense (h^2B), genetic advance (GA) and genetic advance as percent of mean for characters studied at Jimma, 2013/14.

Character	σ^2g	σ^2e	σ^2p	PCV	GCV	h^2B	GA	GAM
SU	0.307	0.15	0.457	28.64	23.48	67.18	0.94	39.64
LL	2.46	1.4	3.86	8.09	6.46	63.73	2.58	10.62
LW	2.34	1.5	3.84	8.65	6.75	60.94	2.46	10.86
PLC	23.49	14.04	37.52	9.31	7.36	62.58	7.90	12.00
PLlg	13.5	8.88	22.37	10.38	8.06	60.31	5.88	12.89
PH	21.03	15.91	36.94	10.79	8.14	56.92	7.13	12.65
COL	0.525	1.43	1.955	13.47	6.98	26.85	0.77	7.45
CLD	0.135	0.17	0.305	10.64	7.08	44.26	0.50	9.70
NCL	4.79	4.9	9.69	26.49	18.64	49.43	3.17	26.98
CML	0.235	0.48	0.715	10.65	6.11	32.87	0.57	7.21
CMD	0.17	0.59	0.76	10.53	4.98	22.37	0.40	4.85
COW	0.0073	0.008	0.0153	27.24	18.81	47.68	0.12	26.75
CMW	0.0025	0.0014	0.0035	26.42	21.13	63.96	0.08	34.82
CMDM	3.16	4.09	7.25	9.64	6.36	43.59	2.42	8.65
CODM	3.385	4.46	7.845	10.14	6.66	43.15	2.49	9.02
Tot. Yi	0.0146	0.0137	0.0284	24.40	17.54	51.68	0.18	25.98

SU = number of suckers per plant, LL = lamina length (cm), LW = lamina width (cm), PLC = plant canopy diameter(cm), PTLg = petiole length (cm), Ph = plant height (cm), COL = cormel length (cm), CLD = cormel diameter (cm), NCL = number of cormels per plant, CML = corm length (cm), CMD = corm diameter (cm), COW = cormel fresh weight per plant (kg), CMW = corm fresh weight per plant (kg), CMDM = corm dry matter content (%), CODM = cormel dry matter content (%), TotYi = total root yield per plant (kg)

variations indicate the presence of high genetic variability for different traits and less influence of environment.

The result is in agreement with Choudhary et al. (2013) which reported high value of phenotypic coefficient of variation along with genotypic coefficient of variation for number of suckers per plant and cormel yield on taro genotypes. Paul and Bari (2012) reported high PCV along with high GCV for cormel weight, corm weight, number of cormels per plant and total fresh weight per plant for tannia genotypes. Similarly, Cheema et al. (2006) reported high PCV and GCV for number of cormels per plant, total yield per plant and corm fresh weight on taro genotypes.

The estimate of broad sense heritability values ranged from 22.37 to 67.18% (Table 4). According to Verma and Agarawal (1982) heritability values greater than 50% are considered as high, values between 20 to 50% as medium and values less than 20 are as low heritable. Based on this, lamina length (63.73%), lamina width (60.94%), number of suckers per plant (67.18%), plant canopy diameter (62.58%), petiole length (60.31%), plant height (56.92%), corm weight (63.96%) and total yield per plant (51.68%) exhibited high heritability estimates, suggesting that, greater effectiveness of selection and improvement to be expected from these characters in future breeding program.

This is in support of Sedeek et al. (2009) who stated that selection would be effective when major portion of variability for different traits in the source population is heritable. This is because there would be a close correspondence between the genotype and phenotype due to relatively small contribution of the environment to the phenotype. Also moderate heritability estimate of cormel diameter (44.26%), cormel fresh weight (47.68%), number of cormels per plant (49.43%), corm dry matter (43.59%), cormel dry matter (43.15%), cormel length (26.85%), corm diameter (22.37%) and corm length (32.87%) exhibited, which indicates the presence of more environmental effect, because of this selection may be difficult due to the masking effect of the environment on germplasm.

Heritability estimates along with genetic advance are normally more helpful in predicting the gain under selection than heritability estimates alone (Bisne et al., 2009). The expected genetic advance expressed as a percentage of the mean (GAM) ranged from 4.85% for corm diameter to 39.64% for number of suckers per plant (Table 4). This indicated that selecting the top 5% of the base population could result in an advance of 4.85 to 39.64% over the population mean.

According to Johnson et al. (1955) genetic advance as percent of mean could be considered as low if it ranges between 0 and 10%, as moderate 10 and 20% and high if it becomes above 20%. Consequently, number of sucker per plant, number of cormel per plant, cormel weight, corm weight and total yield per plant had high genetic advance along with high heritability. Lamina length, lamina width, plant canopy diameter, petiole length and

plant height showed moderate genetic advance along with high heritability. The presence of high heritability coupled with moderate to high genetic advance indicates that these are more inherited traits and most likely the heritability was due to additive gene effects of these characters. Hence, selection for these characters is likely to be more effective and have a greater scope of improvement. This is in support of Johnson et al. (1955) who stated that high heritability along with high genetic advance is more reliable than heritability alone in predicting the results of selection.

Low values of genetic advance were recorded for corm diameter (4.85%), corm length (7.21%), cormel diameter (9.70%) corm dry matter content (8.65%), cormel dry matter content (9.02%) and cormel length (7.45%). These low genetic advances arise from moderate heritability and low estimate of genotypic variances. Therefore, it is imperative that selection of genotypes based on phenotypic performance for these characters would not be effective for improvement. Low genetic advance as part of mean with moderate heritability suggested the role of non-additive gene action (dominance and epistasis) for the control of these characters and most of the variation for these traits were environmental.

This finding is in harmony with that of Singh et al. (2003) who reported high heritability estimates along with high genetic advance for weight of corm per plant, number of cormels per plant and cormels fresh weight per plant among taro germplasms. Similarly, Yadav et al. (2007) and Cheema et al. (2006) reported high heritability along with high genetic advance for number of cormels per plant, corm weight per plant, cormel fresh weight per plant and total yield in taro genotypes. Also Paul and Bari (2012) reported high genetic advance as a percent of mean along with high to moderate heritability for number of cormels per plant, lamina length, lamina width, petiole length, plant height, cormel fresh weight, corm weight and total yield per plant; and also low genetic advance for corm length of tannia germplasm.

In general, the study has shown that there is a wide genetic variability between genotypes of tannia for further utilization in tannia improvement program. However, the result of the present investigation may vary with location and season since this study was conducted in single environment for one season. That means, the available genotypes should be further studied with due emphasis on quantitative characters required to determine further variations and observe the presence and magnitude of genotype-environment interaction. Furthermore, the presence of morphological variation between genotypes is not a guarantee for high genetic variation. Hence, molecular or biochemical studies need to be considered as complementary to this study. Since simple selection of superior types among the existing genotypes could result in identification of promising lines, tannia accessions from other growing areas of Ethiopia need to be collected, so as to broaden the base of existing breeding program. In addition way of inducing flowering and seed propagation, Calcium oxalate content of corm and cormel, effect of time of planting and harvesting, also effect of type of planting material on yield and dry matter content should be considered as future line of work.

Conflict of Interest

The authors have not declared any conflict of interest.

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Mathematical modeling and thermodynamic properties for drying soybean grains

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The aims of this work were to adjust different mathematical models to experimental data describing the drying of the Valiosa cultivar soybean grain, to determine and to evaluate the effective diffusion coefficient and to obtain the activation energy and the thermodynamic properties of the drying process under different air conditions. The Valiosa cultivar soybean grains, with an initial moisture content on a dry basis of 0.56 (d.b., decimal), were dried in an oven with forced air ventilation at five different temperatures (40, 55, 70, 85 and 100°C) until reaching a moisture content of 0.133 ± 0.019 (d.b.). Of the models analyzed, Page's model was selected to best represent the drying phenomenon. The effective diffusion coefficient of soybeans increased with the air temperature and was described by the Arrhenius equation; an activation energy of 22.77 kJ mol^{-1} was reported for liquid diffusion in the drying of the soybeans. The enthalpy and entropy decreased with increasing temperature, while the Gibbs free energy increased with increasing drying temperature.

Key words: *Glycine max*, liquid diffusivity, enthalpy, entropy, Gibbs free energy.

INTRODUCTION

The soybean (*Glycine max*) is the most commonly grown oilseed in the world because of its high protein content, which is important for the diets of humans and animals raised for human consumption (Carvalho, 2012). The soybean culture is one of the most important cultures in Brazil, as it corresponds to 40% of the total grain produced in the country and 27% of the grain produced worldwide. Brazil is the second largest producer and the largest exporter of soybeans, and this culture account for 20% of exports in Brazilian agribusiness (Vernetti and Vernetti Junior, 2009).

The purpose of drying agricultural products is to ensure their quality, as the reduction in the moisture content reduces the biological activity, chemical and physical changes that occur The study of drying provides information on the heat and mass transfer that occur between the biological material and the drying element (usually heated or non-heated atmospheric air), which is crucial for the design, operation and simulation of drying systems and dryers (Corrêa et al., 2003). The use of mathematical models to simulate the drying process in dryers that operate at high during storage. The study of drying provides information on the heat and mass transfer that occur between the biological material and the drying element (usually heated or non-heated atmospheric air), which is crucial for the design, operation

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and simulation of drying systems and dryers (Corrêa et al., 2003). The use of mathematical models to simulate the drying process in dryers that operate at high temperatures is an important tool for engineers who work in the field of drying and storage of grains.

The liquid diffusion theory has been widely used to study the drying of vegetable products. According to Corrêa et al. (2006), the liquid diffusion mechanism is complex because of the diversity in chemical compositions and physical structures of the product. Water diffusion in agricultural products involves different mechanisms, including molecular diffusion, capillary diffusion, surface diffusion, hydrodynamic flow, vapor diffusion and thermal diffusion (Goneli et al., 2009).

Knowledge of the thermodynamic properties involved in the drying of agricultural products allows engineers to design better drying equipment, to calculate energy requirements necessary for the process, to study the properties of adsorbed water, to evaluate the microstructure of food and to study the physical phenomena that occur at the material surface (Corrêa et al., 2010). The aims of this study was to obtain, model the drying curves, evaluate the liquid diffusion and the thermodynamic properties of Valiosa cultivar soybean grains dried under different air-drying conditions.

MATERIALS AND METHODS

The experiments were conducted in the Laboratory for the Postharvest of Vegetables Products (Laboratório de Pós-colheita de Produtos Vegetais) at the Federal Institute of Education, Science and Technology of Goiás (Instituto Federal de Educação, Ciência e Tecnologia Goiano – Câmpus Rio Verde) with Valiosa cultivar soybean grains from the municipality of Santa Helena de Goiás (GO). The initial moisture content of the grains was 0.56 (d.b., decimal); these grains were dried in an oven with forced air ventilation at five different temperatures (40, 55, 70, 85 and 100°C), leading to relative humidities of 25.3, 12.5, 5.7, 3.5 and 2.2%, respectively. The drying continued until the grain moisture content reached 0.133 ± 0.019 (d.b.), determined at 105 ± 1°C for 24 h in three replicates (Brasil, 2009). The reduction in the moisture content during drying was monitored with the gravimetric method (mass loss) using an analytical balance with a resolution of 0.01 g installed on the outside of the oven; knowing the initial moisture content of the product, the drying continued until the desired moisture content was achieved.

The temperature and relative humidity of the external ambientenvironment of the drying chamber were monitored with a psychrometer, using a thermometer with dry bulb and another with wet bulb, and the internal temperature was monitored with a thermometer installed inside the oven. The relative humidity of the drying air was obtained by means of the basic principles of psychrometry, using the free software GRAPSI.

The moisture content ratios of the soybeans during drying were determined with the following expression:

$$RX = \frac{X - X_e}{X_i - X_e} \quad (1)$$

where, RX: ratio of the moisture content of the product, dimensionless; X: moisture content of the product (d.b., decimal); X_i: initial moisture content of the (d.b., decimal); and X_e: equilibrium moisture content of the product (d.b., decimal).

The equilibrium moisture content of the soybeans at each temperature was obtained with the modified version of Henderson's equation, as reported by ASAE (1988). The experimental data describing the drying process of the soybeans were adjusted with mathematical models commonly used to represent the drying of agricultural products; these models are presented in Table 1.

The mathematical models were fitted using nonlinear regression with the Gauss-Newton method using software Statistica 7.0. The models were selected according to the determination coefficient (R^2 in %) and the relative average error (P in %). A relative average error of less than 10% was considered a criterion for model selection, as recommended by Mohapatra and Rao (2005).

$$P = \frac{100}{N}\sum\frac{\left|Y - \hat{Y}\right|}{Y} \quad (2)$$

where, Y: experimental value; $\hat{Y}$: value calculated by the model; N: number of experimental observations.

The liquid diffusion model for the spherical geometric form with the approximation of eight terms (14) was fitted to the experimental data describing soybean drying according to the expression of Brooker et al. (1992):

$$RX = \frac{X - X_e}{X_i - X_e} = \frac{6}{\pi^2}\sum_{n=1}^{\infty}\frac{1}{n^2}\exp\left[-\frac{n^2\cdot\pi^2\cdot D\cdot t}{R_e{}^2}\right] \quad (3)$$

where, RX: ratio of the moisture content of the product, dimensionless; t: time, s; n: number of terms; D: liquid diffusion coefficient, $m^2\ s^{-1}$; and R_e: equivalent radius, m (2.95×10^{-3} m).

The volume of each grain (V_g) was obtained by measuring the three orthogonal axes (length, width and thickness) of fifteen grains at the end of drying with a digital caliper Mitutoyo with a resolution of 0.01 mm, according to the expression proposed by Mohsenin (1986):

$$V_g = \frac{\pi\cdot A\cdot B\cdot C}{6} \quad (4)$$

where, V_g: grains volume, mm^{-3}; A: length, mm; B: width, mm; and C: thickness, mm.

The relationship between the effective diffusion coefficient and the increase in drying air temperature was described with the Arrhenius equation satisfactorily by many researchers (Sousa et al., 2011; Costa et al., 2011; Oliveira et al., 2012; Siqueira et al., 2012), according to the following expression:

$$D = D_o\cdot\exp\left(\frac{-E_a}{R\cdot T_{ab}}\right) \quad (5)$$

where, D_o: pre-exponential factor; E_a: activation energy, kJ mol^{-1}; R: universal gas constant, 8.134 kJ $kmol^{-1}\ K^{-1}$; and T_{ab}: absolute temperature, K.

The thermodynamic properties of the drying of soybean grains were obtained with the method reported by Jideani and Mpotokwana (2009):

$$\Delta H = E_a - R\cdot T \quad (6)$$

$$\Delta S = R\cdot\left(\ln k - \ln\frac{k_B}{h_p}\right) - \ln T_{ab} \quad (7)$$

Table 1. Mathematical models used to predict the drying of agricultural products.

Model equation	Model	
$RX = 1 + at + bt^2$	Wang and Singh (Wang and Singh, 1978)	(2)
$RX = \exp(-k \cdot t^n)$	Page (Page, 1949)	(3)
$RX = \exp(-k \cdot t)$	Newton (Lewis, 1921)	(4)
$RX = a \cdot \exp(-k \cdot t) + c$	Logarithmic (Yagcioglu et al., 1999)	(5)
$RX = a \cdot \exp(-k \cdot t)$	Henderson and Pabis (Henderson and Pabis, 1961)	(6)
$RX = a \cdot \exp(-k \cdot t^n) + b \cdot t$	Midilli (Midilli et al., 2002)	(7)
$RX = a \cdot \exp(-k \cdot t) + (1-a)\exp(-k \cdot a \cdot t)$	Two exponential terms (Sharaf-Eldee et al., 1980)	(8)
$RX = a \cdot \exp(-k_o \cdot t) + b \cdot \exp(-k_1 \cdot t)$	Two Terms (Henderson, 1974)	(9)
$RX = a \cdot \exp(-k \cdot t) + (1-a) \cdot \exp(-k \cdot b \cdot t)$	Diffusion Approximation (Kassem, 1988)	(10)
$RX = a \cdot \exp(-k \cdot t) + (1-a)\exp(-k_1 \cdot t)$	Verma (Verma et al., 1985)	(11)
$RX = \exp\left(\left(-a - \left(a^2 + 4 \cdot b \cdot t\right)^{0,5}\right) / 2 \cdot b\right)$	Thompson (Thompson et al., 1968)	(12)

where, t: drying time, h; k, k_o, k_1: drying constants h^{-1}; and a, b, c, n: models coefficients.

$$\Delta G = \Delta H - T_{ab} \cdot \Delta S \quad (8)$$

where, ΔH = enthalpy, J mol^{-1}; ΔS = entropy, J mol^{-1}; ΔG = Gibbs free energy, J mol^{-1}; k_B = Boltzmann constant, 1.38 x 10^{-23} J K^{-1}; and h_p = Planck constant, 6.626 x 10^{-34} J s^{-1}.

RESULTS AND DISCUSSION

The average values of the moisture content ratio of the soybean grains dried under different air conditions are shown in Table 2. The times required for the grains to reach the moisture content of 0.133±0.019 (d.b.) were 18.6, 11.6, 7.7, 5.9 and 4.7 h for the drying temperatures 40, 55, 70, 85 and 100°C, respectively.

The increase in air temperature was found to cause a reduction in the grain drying time. The reduction in drying time is related to the greater difference between the partial pressure of water vapor in the drying air and in the product caused by the increase in temperature. This greater difference promotes an easier and more rapid water removal; similar observations were made by other authors for numerous products (Resende et al., 2008; Almeida et al., 2009; Sousa et al., 2011; Costa et al., 2011; Oliveira et al., 2012).

The values of the determination coefficient (R^2) and the relative average error (P) of the eleven models adjusted during the drying of the soybeans at different temperatures are shown in Table 3. The determination coefficient (R^2) was above 99% for all models and all drying temperatures, indicating, according to Madamba et al. (1996), a satisfactory representation of the phenomenon under study.

The models presented relative average error values (P) less than 10% for the five conditions analyzed, indicating, according to Mohapatra and Rao (2005), that they provide suitable representations of the drying phenomenon. However, the Wang and Sing (2), Newton (4), Exponential of Two Terms (8) and Thompson (12) models had the highest values of P. The analyzed models satisfactorily represented the process of soybean grain drying; however, the Page (3), Logarithmic (5) and Midilli (7) models had the best overall fits. Thus, due to its simplicity, the Page model was selected to represent the phenomenon of soybean grain drying.

Figure 1 shows the drying curves for soybean at the different studied temperatures generated from the experimental data and the values estimated by the Page model. A satisfactory adjustment of the model to the experimental values obtained over the drying of soybeans.

The drying time of the product was inversely proportional to the temperature; in other words, the higher the temperature, the shorter the drying time. The drying times for 40 and 100°C were 18.6 and 4.7 h, respectively. Corrêa et al. (2010) reported that the reduction in the moisture content of agricultural products, especially of grains and seeds, occurs with decreasing order with increasing temperature due to the difference in the surface moisture and the size of the whole grain. Sousa et al. (2011) and Oliveira et al. (2012) reported similar results for the drying of forage turnip seeds and corn, respectively.

Table 4 shows the values of the coefficients “k” and “n” of the Page model fitted to experimental data describing the kinetics of soybean grain drying at different temperatures.

The magnitude of the drying constant k for the Page model represents the phenomenon whereby temperature increases in the drying air result in increasingly favorable external drying conditions. However, the coefficient n of the Page model was not affected by drying temperature.

Table 2. Moisture content ratio (RX) of Valiosa cultivar soybean grains during drying time (hours) under five temperature conditions (°C).

Temperature (°C)									
40		55		70		85		100	
Time	RX	Time	RX	Time	RX	Time	RX	Time	RX
0.00	1.00	0.00	1.00	0.00	1.00	0.00	1.00	0.00	1.00
0.67	0.97	0.42	0.96	0.25	0.97	0.22	0.97	0.18	0.97
1.30	0.92	0.78	0.92	0.60	0.92	0.48	0.92	0.40	0.90
2.00	0.86	1.13	0.88	0.93	0.87	0.65	0.88	0.60	0.85
2.40	0.84	1.52	0.84	1.20	0.83	0.85	0.84	0.77	0.81
3.02	0.79	1.83	0.81	1.45	0.79	1.08	0.80	0.93	0.77
3.58	0.76	2.22	0.77	1.68	0.76	1.37	0.75	1.10	0.72
4.37	0.71	2.60	0.74	1.92	0.73	1.60	0.72	1.25	0.69
4.85	0.68	2.98	0.70	2.17	0.70	1.80	0.68	1.42	0.66
5.45	0.65	3.37	0.67	2.48	0.66	2.02	0.65	1.63	0.62
6.43	0.61	3.83	0.64	2.78	0.63	2.25	0.61	1.85	0.58
7.05	0.58	4.32	0.60	3.17	0.59	2.48	0.58	2.05	0.55
7.72	0.55	4.78	0.57	3.52	0.55	2.70	0.55	2.23	0.51
8.55	0.52	5.25	0.54	3.87	0.52	2.97	0.51	2.43	0.47
9.58	0.48	5.82	0.50	4.25	0.48	3.20	0.48	2.65	0.44
10.50	0.45	6.27	0.48	4.63	0.45	3.55	0.44	2.90	0.41
11.53	0.42	6.80	0.45	4.97	0.42	3.85	0.41	3.13	0.38
12.62	0.38	7.50	0.42	5.33	0.39	4.08	0.39	3.35	0.35
13.55	0.35	8.05	0.39	5.65	0.36	4.32	0.36	3.55	0.32
14.60	0.32	8.70	0.36	6.10	0.33	4.60	0.33	3.77	0.30
15.68	0.29	9.45	0.33	6.62	0.30	4.88	0.30	3.97	0.27
16.55	0.26	10.00	0.31	6.95	0.28	5.25	0.28	4.17	0.25
17.38	0.24	10.85	0.28	7.30	0.26	5.58	0.25	4.38	0.23
18.58	0.21	11.57	0.25	7.67	0.24	5.87	0.23	4.75	0.21

Table 3. Determination coefficient (R^2, %) and relative average error (P, %) for the models analyzed during the drying of soybean grains under various temperature conditions (°C).

Model	Temperature (°C)									
	40		55		70		85		100	
	R^2	P	R^2	P	R^2	P	R^2	P	R^2	P
2	99.75	2.41	99.92	1.18	99.97	0.55	99.97	0.46	99.96	0.68
3	99.88	1.69	99.98	0.43	99.98	0.68	99.99	0.48	99.96	0.83
4	99.84	1.78	99.96	0.49	99.62	0.68	99.39	3.47	99.58	3.27
5	99.91	1.48	99.98	0.39	99.98	0.38	99.97	0.61	99.97	0.74
6	99.88	1.70	99.98	0.39	99.82	1.89	99.72	2.21	99.78	2.11
7	99.93	1.19	99.98	0.41	99.99	0.39	99.99	0.31	99.97	0.68
8	99.84	1.78	99.96	0.49	99.62	2.80	99.98	0.39	99.58	3.27
9	99.89	1.64	99.98	0.39	99.82	1.89	99.72	2.21	99.97	0.71
10	99.89	1.63	99.96	0.49	99.97	0.46	99.94	0.90	99.96	0.71
11	99.89	1.67	99.97	0.41	99.97	0.46	99.94	0.90	99.95	0.80
12	99.84	1.78	99.96	0.49	99.62	2.80	99.39	3.47	99.58	3.27

Thus, for the range of temperatures studied, the drying of soybean grains can be estimated using the following expression:

$$RX = \exp\left(-\left(-0.0702 + 0.0033T\right) \cdot t^{1.0764}\right) \quad (9)$$

where: T: drying temperature (°C); t: drying time (h).

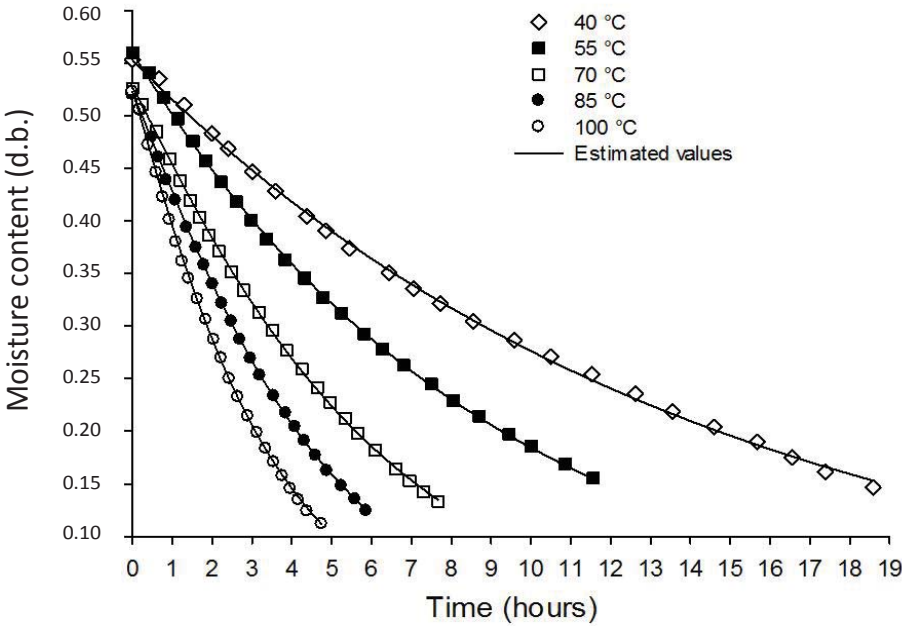

Figure 1. Drying curves, experimental data and values estimated by Page model for the soybean grains at temperatures of 40, 55, 70, 85 and 100°C.

Table 4. Coefficients of the Page model adjusted for different drying conditions of Valiosa cultivar soybean grains.

Coefficient	Temperature (°C)					
	40	55	70	85	100	Average values
k	0.0727***	0.1138***	0.1505*****	0.1962**	0.2813**	K = -0.0702+0.0033T
n	1.0327***	1.0178***	1.0981*****	1.1311**	1.1022**	1.0764

**Significant at 1% by test t.

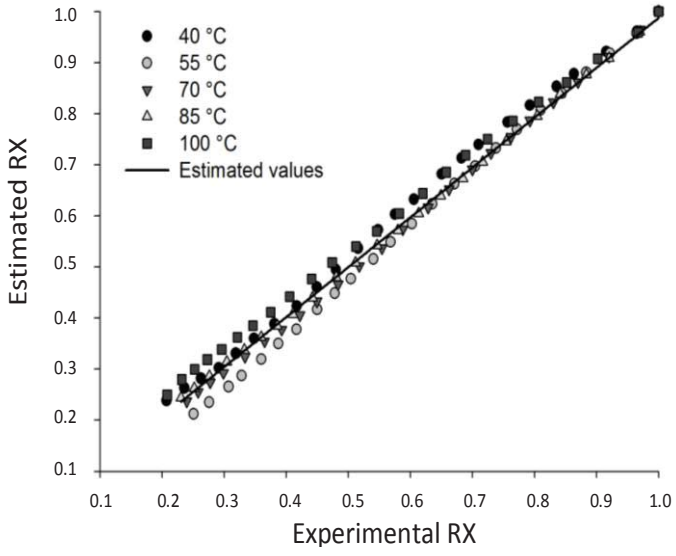

Figure 2. Experimental and estimated values of the moisture content ratio obtained using the Page model as a function of soybean drying temperature.

Figure 2 shows the experimental and the estimated data for the moisture content ratio (RX) obtained using the Page model, the values obtained using Equation 9 and the values presented in Table 2. This model provided a good adjustment to the data while adequately describing the process of soybean grain drying. The reduction in the moisture content ratio leads to a greater discrepancy between the estimated and experimental values.

The values of the effective diffusion coefficient of soybeans as a function of the conditions of the drying air are shown in Figure 3. The effective diffusion coefficient increased linearly with increasing temperature of the drying air, with values of 0.847×10^{-11} to 3.46×10^{-11} $m^2 s^{-1}$ for temperatures ranging from 40 to 100°C, indicating a greater intensity of water transport from the inside to the periphery of the grain and corroborating results obtained by Mohapatra and Rao (2005), Resende et al. (2008), Almeida et al. (2009), Costa et al. (2011), Corrêa et al. (2011) and Siqueira et al. (2012). Madamba et al. (1996) reported that the effective diffusion coefficients were on the order of 10^{-11} to 10^{-9} $m^2 s^{-1}$.

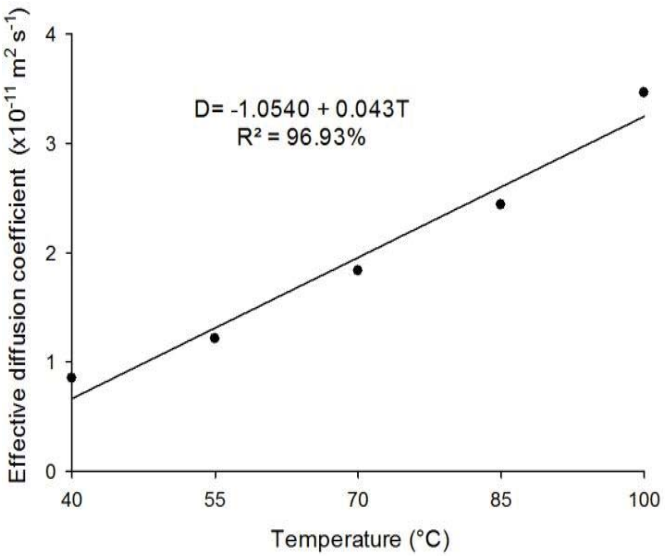

Figure 3. The values of the effective diffusion coefficient (m^2 s^{-1}) obtained for soybean grains drying at temperatures of 40, 55, 70, 85 and 100°C.

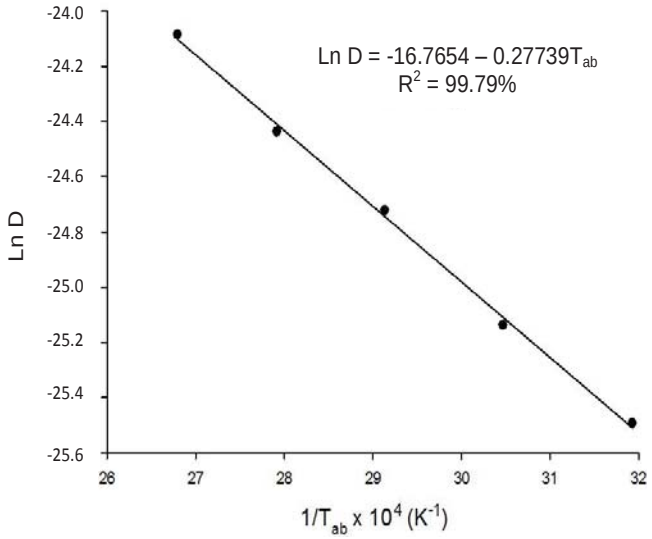

Figure 4. Arrhenius representation for the effective diffusion coefficient for soybean drying at temperatures of 40, 55, 70, 85 and 100°C.

Sousa et al. (2011) studied the drying of fodder radish seeds and obtained values similar to those found in the present work on the order of 3.23×10^{-11} to 10.42×10^{-11} $m^2.s^{-1}$ at temperatures of 30 and 70°C, respectively. Gely and Santalla (2007) and Oliveira et al. (2012) encountered values on the order of 1.18×10^{-12} to 6.76×10^{-12} $m^2.s^{-1}$ and 1.54×10^{-13} to 4.85×10^{-13} $m^2.s^{-1}$ $m^2.s^{-1}$ for the diffusion coefficients of quinoa seeds and corn grains, respectively. Thus, water is removed more rapidly from soybeans than from quinoa seeds and corn grains.

The dependence of the effective diffusion coefficient of the soybean grains on the drying air temperature was represented by the Arrhenius expression as illustrated in Figure 4. The activation energy is defined as the ease with which water molecules overcome the energy barrier for migration from the interior of the product to its exterior (Resende et al., 2005). In the present work, the activation energy for the liquid diffusion of soybeans was 22.77 $kJ.mol^{-1}$ in the studied temperature range. According to Zogzas et al. (1996), the activation energy for agricultural products ranges from 12.7 to 110 $kJ.mol^{-1}$; thus, the value obtained in the present study is within this range.

Kitic and Viollaz (1984) obtained a value close to that found in the present work. These authors reported an activation energy for the soybean of 28.80 $kJ.mol^{-1}$. Gely and Santalla (2007) and Costa et al. (2011) evaluated the drying of quinoa and crambe and reported activation energies of 37.97 and 37.07 $kJ.mol^{-1}$, respectively. The activation energy of soybean encountered in the present study was lower than that found in other studies; this may be due to the more unstable bond between water and the product evaluated, as reported by Siqueira et al. (2012).

Table 5 shows the values of enthalpy, entropy and Gibbs free energy for the different drying conditions. The enthalpy and entropy decreased while the Gibbs free energy increased linearly with increasing drying temperature.

The enthalpy is related to the energy required to remove water from the product during the drying process; thus, the enthalpy decreases with increasing drying temperature (Oliveira et al., 2010). This behavior was observed for soybean grains during the reduction of the moisture content, indicating that lower temperatures require more energy.

According to Goneli et al. (2010), the entropy is a thermodynamic property that can be related to the degree of disorder between the water and the product. The entropy decreases with increasing drying air temperature. Corrêa et al. (2010) reported that this behavior is expected because the decrease in drying temperature decreases the excitation of water molecules of the products and increases the order of the water-product system.

The Gibbs free energy is related to the work required to produce available sorption sites (Nkolo Meze'e et al., 2008). The Gibbs free energy can be positive for endogenous reactions where it is necessary to add energy from the environment. The Gibbs free energy can be negative when the phenomenon occurs spontaneously without the addition of energy. The Gibbs free energy of soybean grains was found to be positive and increased with increasing drying temperature. This behavior was also observed by Corrêa et al. (2011) when studying the thermodynamic properties of corn cobs drying at temperatures of 45, 55 and 65°C.

Equations used to determine the enthalpy, the entropy and the Gibbs free energy for the temperature range

Table 5. Values of enthalpy (ΔH, J mol^{-1}), entropy (ΔS, J mol^{-1} K^{-1}) and Gibbs free energy (ΔG, J mol^{-1}) for different conditions of drying air used for Valiosa cultivar soybean grains.

Thermodynamic properties	Temperature (°C)					Equation	R^2 (%)
	40	55	70	85	100		
ΔH	20164	20040	19915	19790	19666	ΔH = 20496.9-8.3T	99.9
ΔS	-267.1	-263.8	-261.8	-259.9	-257.3	ΔS = -272.9-0.16T	98.8
ΔG	103811	106596	109758	112900	115684	ΔG = 95727+200.3T	99.9

studied can be found in Table 5. These thermodynamic properties behaved in a linear manner and were characterized by high coefficients of determination.

Conclusions

All of the analyzed models satisfactorily represented the drying of grains; however, the Page model, due to its simplicity, was chosen to best represent the phenomenon. The effective diffusion coefficient of soybeans increased with increasing drying air temperature; this phenomenon was described by the Arrhenius equation and characterized by an activation energy of 22.77 $kJ.mol^{-1}$.

The enthalpy and entropy decreased with increasing drying temperature and the entropy was negative at all temperatures studied. The Gibbs free energy was positive for the analyzed conditions and increased with increasing drying temperature.

Conflict of Interest

The authors have not declared any conflict of interest.

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