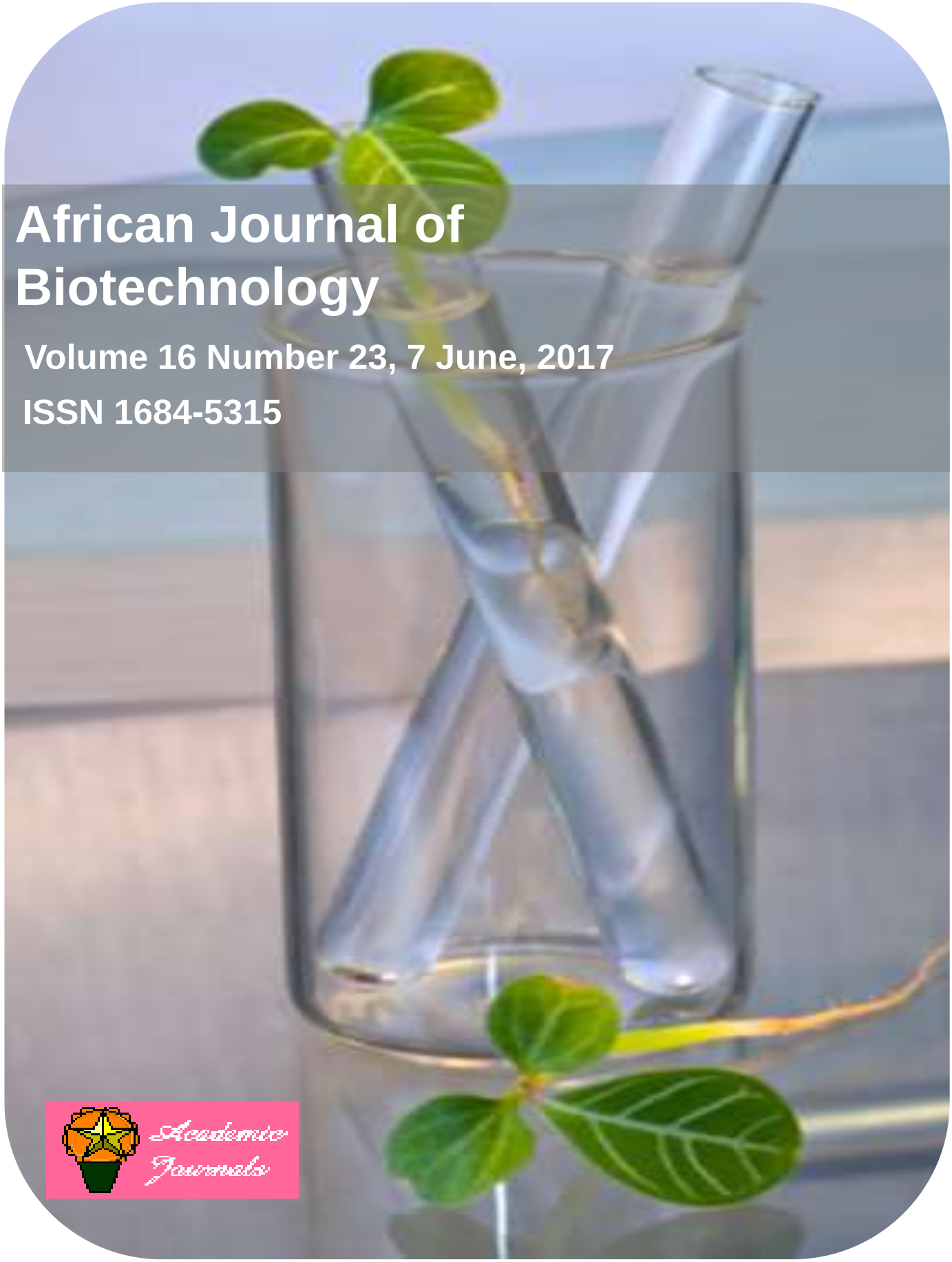

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Full Length Research Paper

Agrobacterium-mediated genetic transformation of Fonio (*Digitaria exilis* (L.) Stapf)

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Fonio (*Digitaria exilis)* is a crop grown in Africa for its excellent culinary and nutritional properties and is a valuable source of income for smallholder farmers. Its improvement has long been neglected by scientists because the crop was one of the lost crops of Africa. Recent advances in Fonio research are centered on its evolution, nutritional and economic values. The lack of a transformation and other biotechnology protocols developed for Fonio has greatly hindered genetic improvement of this crop. In this study, *Agrobacterium*-mediated genetic transformation was developed for two genotypes of *D. exilis* ('Agyong' and 'Churiwe'). Three-weeks-old callus derived from stem segments were co-cultivated with *Agrobacterium tumefaciens* for 3 days. After selection on Hygromycin B medium, plants were regenerated on Murashige and Skoog (MS) medium supplemented with 0.5 mg l^{-1} BA and 0.1 mg l^{-1} gibberellic acid. Regenerated shoots were rooted on hormone-free MS medium. Histochemical gus assay and polymerase chain reaction (PCR) confirmed the presence of the *gus* gene in transformed tissues. The transformation frequency was 2.1 and 2.7% for "Agyong" and "Churiwe", respectively. The transgenic plants were phenotypically normal. This protocol would provide useful platform for genetic improvement of this crop.

Key words: *Digitaria exilis*. Transfomation efficiency, β-glucuronidase (GUS) assay, *Agrobacterium tumefaciens*.

INTRODUCTION

Fonio (*Digitaria exilis)* is a C4 annual herb that tillers freely. It is a native of West Africa and belongs to the family Poaceae, sub-family Panicoideae and tribe Paniceae. There are two important cultivated species in the genus, namely, *D. exilis* and *Digitaria iburua*. The inflorescence in both species is a panicle with finger-like projections, 2 to 5 in *D. exilis* and 4 to 10 in *D. iburua*. The grains are extraordinarily tiny, measuring 0.5 to 1 mm in diameter and 0.75 to 2 mm in length. The fruit is a caryopsis firmly secured between two husks (lemma and palea); in *D. exilis* the husk is white, hence the name white Fonio, in contrast to *D. iburua* that has dark-brown husk and is commonly called black Fonio. The most variable of all the species is *D. exilis* which is cultivated in

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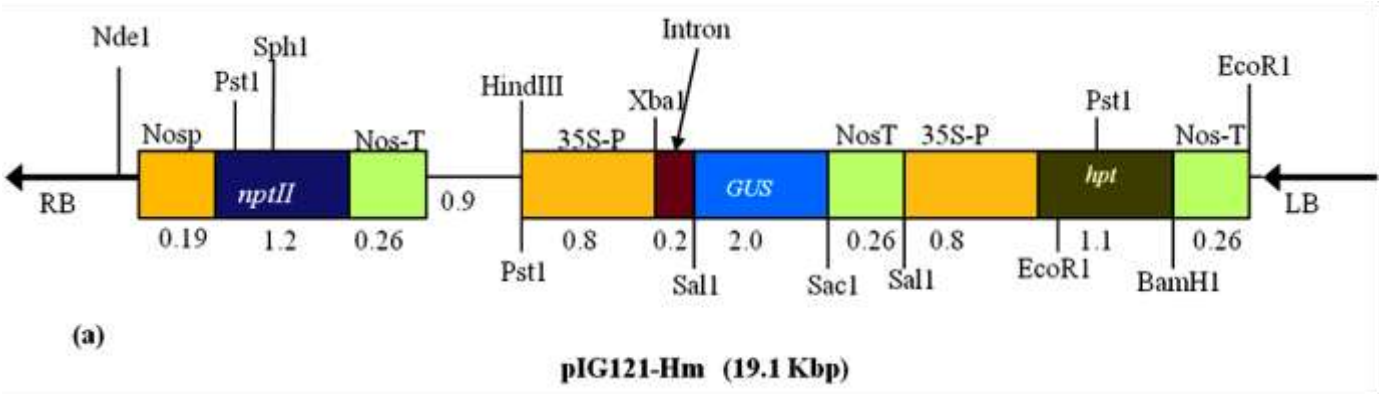

Figure 1. T-DNA map of the construct used for transformation of Fonio. RB: Right border; LB: left border; nptII: neomycin phosphotransferase gene; hpt: hygromycin phosphotransferase; gus: b-glucuronidase gene.

many West African countries where they are called by different names (Ayo and Nkama, 2006; Adoukonou-Sagbadja, 2010).

According to Bennett et al. (2000) and Caponio and Rua (2003), the number of chromosomes in the genus ranges from 2n = 18 (diploid) to 2n = 12x = 108 (dodecaploid).

Fonio seeds have excellent culinary and nutritional properties and are sometimes regarded as the 'grain of life' because they supply food to several millions of people, when the major crops are yet to mature and food is generally scarce. The crop is also used as a valuable source of income, especially for small-scale farmers (Adoukonou-Sagbadja et al., 2007a). According to Kuta et al. (2003), the significant contribution that Fonio could make in resolving food crisis in Africa is expected to rekindle interest in the improvement of this crop. Available literature on the crop centers around genetic characterization of the plant using Random Amplified Polymorphic DNA (RAPD) markers and flow cytometry (Adoukonou-Sagbadja et al., 2007a, b, Adoukonou-Sagbadja, 2010) as well as microsatellite markers (Barnaud et al., 2012). Information is also available on the nutritional and physiochemical property of the crop (Temple and Bassa, 1991; Jideani and Akingbala, 1993; Jideani et al., 1994, 1996; Chukwu and Abdul-Kadir, 2008; Ballogou et al., 2013) as well as cultivation and uses of Fonio (Jideani, 1999).

Although, the use of genetic transformation as a tool for crop improvement and for the development of functional genomics is well documented, literature on *in vitro* cultures or genetic engineering of Fonio is lacking. There is therefore the need to explore the possibility of using genetic transformation for improvement of Fonio so as to increase its productivity. In our previous study, an efficient regeneration protocol for Fonio was developed (Ntui et al., 2010). In the present report, a protocol is given for transformation of *D. exilis* based on *Agrobacterium*-mediated technology.

MATERIALS AND METHODS

Seed germination and callus induction

Seeds of two genotypes of *D. exilis*, 'Agyong' and 'Churiwe' were surface sterilized in 70% ethanol for 5 min and 1.5% (v/v) sodium hypochlorite solution for 20 min before rinsing them thrice in sterile distilled water. The seeds were then germinated and maintained for one month on Murashige and Skoog (1962) medium, containing 10 g l^{-1} sucrose and 8 g/l agar in a growth room at 25±1°C, 16 h photoperiod, 30 to 40 µmol m^{-2} s^{-1} cool white fluorescent light. The pH of the medium was adjusted to 5.8 prior to the addition of agar and autoclaving at 121°C for 15 min.

Stem segments (culm) about 5 mm in length were cut from the four week-old seedlings and cultured on callus induction medium (CIM, which was Murashige and Skoog (MS) medium containing 2 mg/l 2,4-D, 1 g/l casamino acid, pH 5.8 and 8 g /l agar). Cultures were placed at 26 ± 1°C in the dark for three weeks. Stem segments were subcultured to fresh medium after two weeks.

Agrobacterium culture, co-cultivation, selection and plant regeneration

In this study, *Agrobacterium* strain EHA101 (Hood et al., 1986) was used. The *Agrobacterium* carries the plasmid pIG121-Hm (Ohta et al., 1990) which contains hygromycin phosphotransferase (hpt), neomycin phosphotransferase II (nptII), and β-glucuronidase (gus) as reporter genes in T-DNA region (Figure 1). The *Agrobacterium* was cultured overnight in a reciprocal shaker (120 cycles/min) at 28°C in 50 ml liquid LB medium containing 50 mg/l kanamycin sulphate, 25 mg/l hygromycin B and 25 mg/l chloramphenicol. After centrifugation, the bacteria were resuspended to final density of OD_{600} = 0.5 in inoculation medium, which was MS medium containing 68 g/l sucrose, 36 g/l glucose, 3 g/l KCl, 4 g/l $MgCl_2$, pH 5.2 and 100 µM acetosyringone (3,5-dimethoxy-4-hydroxy-acetophenone; Sigma-Aldrich, St. Louis, MO, USA).

Three-week-old calli were dipped in the bacterial suspension for 10 min with gentle shaking. Thereafter, the calli were dried on a Whatman filter paper and co-cultivated in the dark on CIM, pH 5.2 and supplemented with 100 µM acetosyringone for three days.

After co-cultivation, the calli were washed three times with sterilized distilled water containing 10 mg/l meropenem trihydrate (Meropen; Dainippon Sumitomo Pharma, Osaka, Japan) (Ogawa and Mii, 2007) and cultured for 4 weeks on CIM supplemented with 20 mg/l meropenem to kill bacterial carryover and 50 mg/l

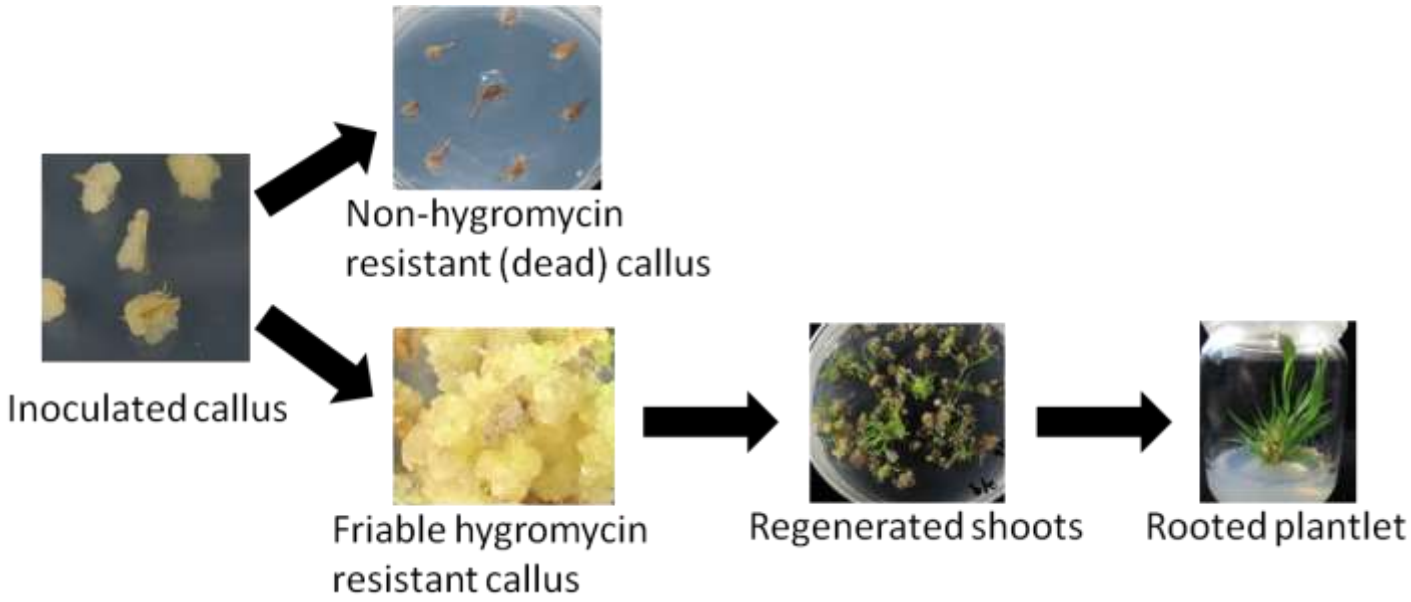

Figure 2. Production of transgenic plants from three month old callus after inoculation with *Agrobacterium* strain EHA 101pIG121-Hm.

Table 1. Transformation efficiencies of two cultivars of *D. exilis* inoculated with *Agrobacterium tumafaciens* EHA101pIG121-Hm.

Cultivar	No. of calli inoculated	No. of plants regenerated	PCR+Plants	Transformation efficiency (%)
Agyong	234	31 (13.25)	5 (16.13)	2.14
Churiwe	219	26 (11.87)	6 (23.1)	2.74

Values in brackets are percentages.

hygromycin B for selecting transformed tissues in the dark. The calli were subcultured bi-weekly into fresh medium of same composition.

After selection, brown or black calli were removed and only creamish healthy calli were transferred to MS medium containing 30 g/l sucrose, 0.5 mg/l BA, 0.1 mg/l gibberellic acid (GA_3), 20 mg/l hygromycin B and 10 mg/l meropenem for shoot regeneration. Regenerated shoots were transferred to hormone-free MS medium containing 20 mg/l hygromycin B and 10 mg/l meropenem.

Gus assay and polymerase chain reaction

Histochemical gus assay (Jefferson et al., 1987) was used to assess gus staining of putative transformed plants using 5-bromo-4-chloro-3-indolyl beta-D-glucuronide (X-Gluc) as the substrate. After selection, a few creamish healthy calli were subjected to gus assay by incubation in sodium phosphate buffer containing X-Gluc for 18 h at 37°C.

For polymerase chain reaction (PCR) analysis, genomic DNA was extracted from leaves of regenerated seedlings using the modified cetyltrimethylammonium bromide (CTAB) method as reported by Rogers and Bendich (1988). PCR was done for the *gus* gene using the following pair of primers: GUS-F: 5′-GGTGGG AAAGCGTTACAAG-3′; GUS-R: 5′-GTTTACGCGTTGCTTCCGCCA-3′.

Transformation efficiency was calculated as the number of PCR positive plants × 100/number of calli inoculated.

RESULTS AND DISCUSSION

Overview of the transformation and regeneration protocol

Two genotypes, ‘Agyong’ and ‘Churiwe’ were selected to develop transformation protocol for Fonio. These genotypes were selected because of their high regeneration efficiency based on our previous study (Ntui et al., 2010). Culm segments were used as the explants, as these were the only tissues which developed calli based on our previous study (Ntui et al., 2010).

Approximately, 80% of the culms developed calli within 3 weeks of culture on callus induction medium. After inoculation with *Agrobacterium* and upon selection on hygromycin medium, some calli turned brownish and died while others remained creamish and produced friable callus (Figure 2). These friable calli together with other hygromycin resistant calli were transferred to regeneration medium. Of the calli that were transferred to regeneration medium, only 13.25 and 11.87%, respectively for Agyong and Churiwe, produced shoots (Table 1). This regeneration frequency was very low and was produced only by friable calli. Regenerated shoots were successfully

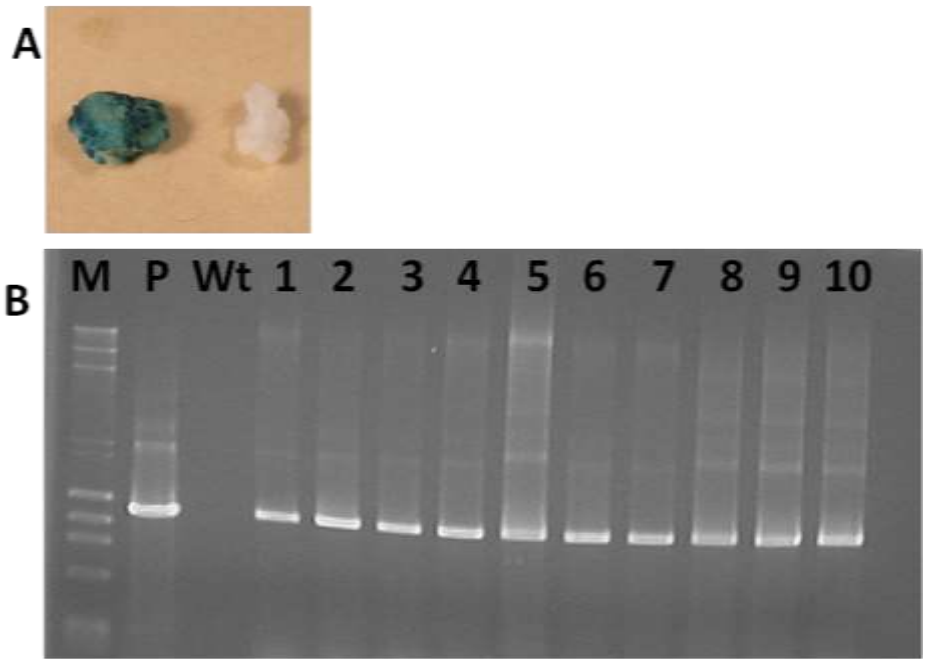

Figure 3. Molecular analysis of transgenic tissues. (A) Detection of Gus activity in callus. (B) PCR amplification of the *gus* gene. M: Molecular size marker (ø174HaeIII digests); P: positive control (plasmid DNA); Wt: wild-type (untransformed control). Lanes 1-10, independent transgenic lines of 'Agyong' (1-5) and 'Churiwe' (6-10).

rooted in hormone-free medium (Figure 2).

Gus assay and PCR analysis

Gus activity was only detected in transgenic tissues but not in the untransformed tissues after using X-Gluc (Figure 3A). From PCR analysis done, it was found that a DNA fragment corresponding to the *gus* gene was amplified from genomic DNA isolated from leaves of putative transformed plants (Figure 3B). The transformation efficiencies obtained were 2.14 and 2.74, respectively for cultivars 'Agyong' and 'Churiwe' (Table 1), which are quite low. The low transformation efficiencies may have resulted from the high number of escapes probably because of insufficient selection regime and also due to the low concentration of hygromycin used in the regeneration medium. In some cereals, hygromycin concentration of up to 100 mg/l has been used to select transgenic plants. In some rice genotypes, 50 mg/l hygromycin is sufficient to select transgenic tissues (Sahoo et al., 2011). Since the sensitivity of callus to hygromycin B was not checked, possibly the right concentration of the antibiotic was not used for the selection. Checking the sensitivity of callus to hygromycin is important to minimize escape. However, as this is not the only factor that affects transformation efficiency of plants, other factors such as *Agrobacterium* strain and concentration, pH, inoculation and co-cultivation time amongst others should be optimized to increase the transformation efficiency of this plant and subsequently facilitate its functional genomics.

Genetic transformation provides a greater potential for overcoming breeding barriers in crop improvement (Wambugu, 1999, 2001; Machuka, 2001). The transformation protocol developed above could prove useful for genetic engineering of Fonio for increased grain size, pest and disease resistance, herbicide tolerance and ability to withstand lodging among others. A number of gene constructs for many of these traits and which are routinely used for cereal transformation are available and can be explored for Fonio genetic engineering by adopting the transformation protocol developed in this study.

Conclusion

In this protocol, it was observed that it took about 10 weeks (the essential steps given in Figure 4) to produce transgenic plants from co-cultivation with *Agrobacterium*. Although, the transformation efficiency is low, this is however, the first report of *Agrobacterium*-mediated genetic transformation of *Digitaria*. The protocol described here will provide useful platform for developing high efficient transformation protocol and for genetic improvement of this important cereal.

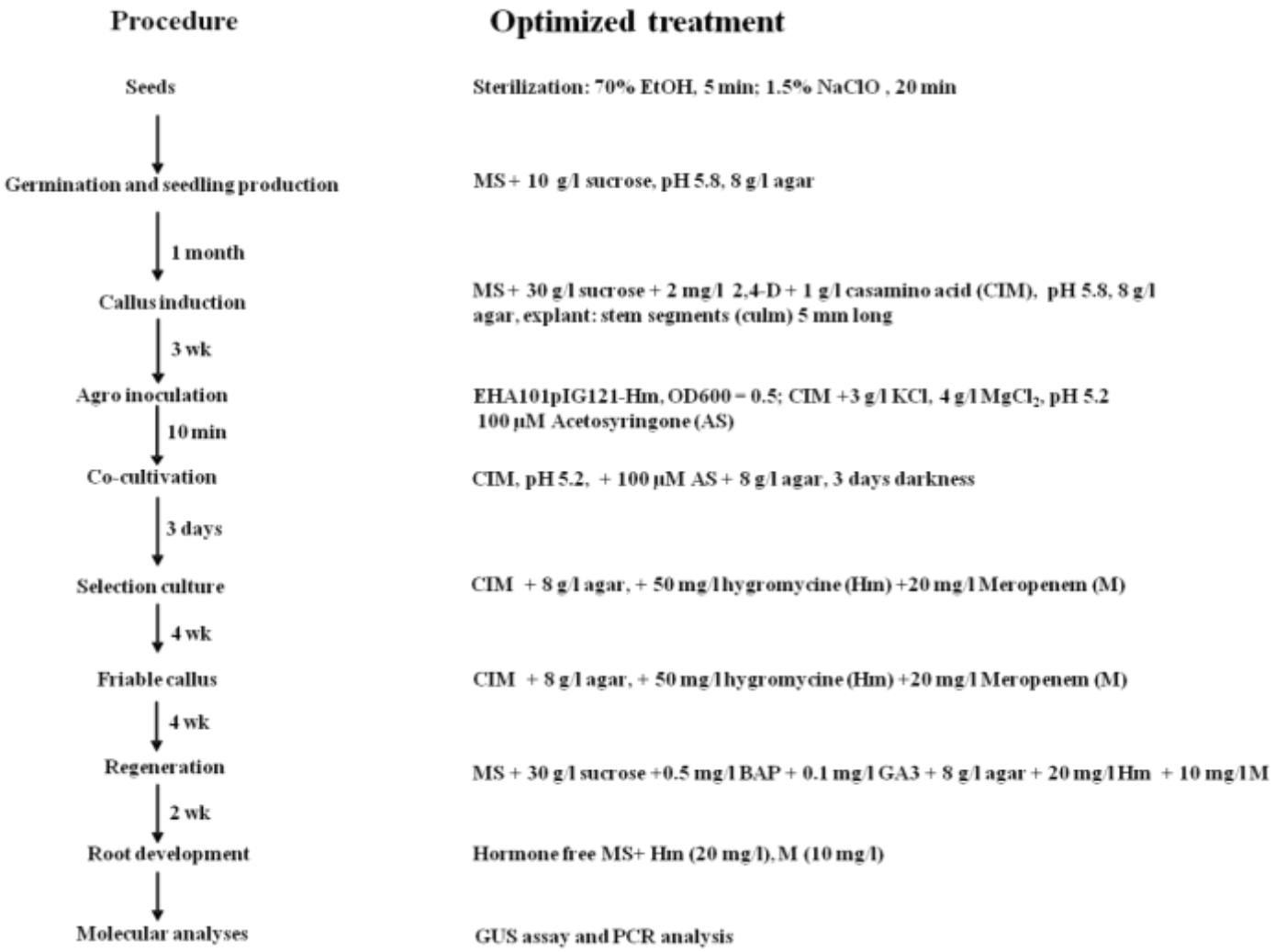

Figure 4. Flow chart for the transformation of Fonio.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Full Length Research Paper

Evaluation of sesame (*Sesamum indicum* L.) germplasm collection of Tamil Nadu for α-linolenic acid, sesamin and sesamol content

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A germplasm collection of Tamil Nadu comprising of nine varieties and six landraces were analyzed for their ω3 fatty acid content, lignans such as sesamin and sesamol. The percentage of ALA ranges from 0.1 to 0.55%. A wide variation was observed in sesamol content. The highest sesamol content of 0.547 mg/g was found in a variety SVPR1. The sesamin content ranged between 2.03 and 6.45 mg/g. A negative relationship was observed between total oil content and ALA. White sesame seeds had higher sesamol content whereas black varieties revealed higher sesamin content.

Key words: ω3 fatty acid, sesame, sesamin, sesamol, Tamil Nadu germplasm.

INTRODUCTION

Sesame (*Sesamum indicum* L.) is an ancient oil yielding crop known as “Queen of Oilseeds” (Nayar, 1984). In the world production, Myanmar ranks first with 6, 20,000 metric tonnes (mt) of sesame while India ranks second with 6, 10,000 mt. In area under production, India ranks first with about 18, 20,000 ha whereas Myanmar stands second with 15, 70,000 ha (FAOSTAT, 2011). In India, Uttar Pradesh, Madhya Pradesh, Rajasthan, Orissa, Gujarat, Andhra Pradesh, Tamil Nadu and Maharashtra are the states which are suitable for sesame cultivation. Chemical composition of sesame seed shows 58% oil, 25% protein, 13.5% carbohydrate and 5% ash. Sesame oil has high percentage of desirable mono 18:1 and poly unsaturated fatty acids 18:2. The fatty acids present in sesame oil are oleic acid (43%), linoleic acid (35 %), palmitic acid (11%) stearic acid (7%) and trace amounts of α-linolenic acid which together comprises of about 96% of total fatty acids (Elleuch et al., 2007). Sesame is also used as nutraceutical and phytoceuticals as it has significant effect on preventing several diseases such as cancer, cardiovascular disease, atherosclerosis and the process of ageing in human being (Suja et al., 2004; Pathak et al., 2014a). Sesame seeds are described as the “seeds of immortality” for its resistance to oxidation and rancidity when stored at ambient temperature (Bedigian and Harlan, 1986; Pathak et al., 2014b).

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Table 1. Details of sesame varieties and landraces used in this study.

S/N	Crop/variety	Parentage	Place of collection	Colour of seed
1	TMV3	South Arcot variety x Malabar variety	Tindivanam	Dark brown
2	TMV4	Pureline selection sattur (local)	Tindivanam	Brown
3	TMV5	Purline selection from Srivaikuntam(local)	Tindivanam	Brown
4	TMV6	Selection from Andhra local	Tindivanam	Brown
5	TMV 7	Si 250 x ES 22	Tindivanam	Brown
6	CO 1	(TMV 3 x Si 1878) x Si 1878	Coimbatore	Dark brow/black
7	SVPR 1	Selection from “Western Ghats White”	Srivilliputhur	White
8	VRI 1	Pureline selection from Tirukattupali local	Vridhachalam	Brown
9	VRI 2	VS9003 X TMV6	Vridhachalam	Reddish brown
10	VS07023	Landraces	Vridhachalam	Brown
11	MD1	Landraces	Madurai	White
12	MD2	Landraces	Madurai	White
13	MD3	Landraces	Madurai	White
14	MD4	Landraces	Madurai	White
15	MD5	Landraces	Madurai	White

Sesamin is a major lignan compound present in sesame seeds. If the intake of sesamin is around 100 to 150 mg, it can preserve vitamin E in the human body. It also acts as a fatty acid metabolism modifier (Wang et al., 2012). Sesamolin is another major lignan present in the sesame seeds which is thermally unstable. During sesame oil processing, sesamolin is converted into sesamol and some other products by heat. Sesamol has very strong antioxidant activity. The sesamol content is very high in roasted sesame seed oil than unroasted sesame seed oil. It acts as a scavenger of free radicals (Singh et al., 2015). The present study was carried out to evaluate the α-linolenic acid (omega 3), sesamin and sesamol content in *Sesamum indicum* L. of Tamil Nadu germplasm for enhancing oil quality and anti-oxidant properties.

MATERIALS AND METHODS

Sesame seeds

Sesame seeds cultivated in Tamil Nadu were collected from Tamil Nadu Agricultural University (TNAU) and oil seed Research stations of TNAU located in Madurai, Coimbatore, Vridhachalam, Srivilliputhur and Tindivanam. Totally 15 accessions were collected. Out of them, nine were varieties and six of them were landraces. The parentage, seed colour and other information regarding the germplasm were collected from TNAU Agritech portal and presented in Table 1.

Lipid extraction

One gram of each seed variety was crushed in a mortar and pestle using liquid nitrogen to make a fine powder. Lipids were extracted from each seed variety with chloroform: methanol (2:1) as described by Bligh and Dyer (1959).

Percentage of oil in ground sample = [Weight of oil (g) / Weight of samples (g)] × 100

Fatty acid methyl esterification

Oil sample of each accession was converted into fatty acid methyl esters (FAME). Lipids were trans esterified with 6% potassium hydroxide in methanol. Lipids were incubated in water bath at 60°C for 3 h. The FAME were dried under nitrogen gas and dissolved with dichloromethane.

Gas chromatography analysis

FAME were analysed on Systronics gas chromatography instrument equipped with capillary column of 30 m × 0.3 mm internal diameter and Flame ionization detector. The oven was programmed to hold the temperature at 100°C and increased up to 200°C/5 min. Nitrogen was used as a carrier gas. The Flame ionization detector and injector temperature was maintained at 260°C. The peak was identified by comparison with the retention time of a commercial standard mixture of FAME (Supelco 37-component FAME mix Sigma, USA). Lipid analysis was performed independently for three times. Values were reported as average and standard deviation (n=3).

Sesamin and sesamol extraction

Sesame seeds were roasted and ground into powder using liquid nitrogen. Methanol was added and centrifuged at 4000 rpm for 5 min by a modified protocol (Rangkadilok et al., 2010). The supernatant was filtered through 0.22 µm nylon membrane filter (Pall Corporation).

HPLC analysis

High performance liquid chromatography was performed to analyze the lignans such as sesamin and sesamol. Sesamol and sesamin content were determined by direct injections using C18 reverse phase column equipped with UV detector at 290 nm. The mobile

Table 2. Total oil content and Alpha linolenic acid content of sesame germplasm in Tamil Nadu.

Sesame varieties	Total oil content (%)	Alpha linolenic acid content (%)
TMV3	51.09	0.17
TMV4	51.55	0.23
TMV5	51.91	0.24
TMV6	55.2	0.18
TMV7	50.15	0.55
CO1	51.56	0.19
SVPR1	51.83	0.13
VRI 1	51.19	0.18
VRI 2	51.75	0.13
VS07023	51.44	0.1
MD1	51.43	0.22
MD2	53.8	0.12
MD3	50.25	0.17
MD4	52.87	0.21
MD5	53.07	0.19

phase for both lignin was methanol: water (80:20) at the flow rate of 0.8 ml/min. Peaks were identified and quantified by comparison with commercial standard Sesamol (Sigma chemical,USA) and Sesamin (Cayman chemical, USA):

$$\text{Sample Concentration (mg/g of seeds)} = \frac{\text{Sample peak area} \times \text{standard concentration}}{\text{Standard peak area}}$$

RESULTS AND DISCUSSION

Total oil content

The total oil content and α-linolenicacid of sesame seeds from Tamil Nadu germplasm were analyzed and given in Table 2. The highest oil content of 55.2% was found in the sesame variety TMV6 whereas TMV7 recorded the lowest of 50.15%. The oil content of TMV7 was low when compared to other varieties of Tamil Nadu whereas TMV6 has recorded with high oil content and low yield of 611 kg/ha compared toTMV7 variety with an average yield of 802 kg/ha. TMV7 is a recently released variety which is also tolerant to root rot disease (Unal and Yalcin, 2008). Similarly, the oil content of four varieties of sesame collected from Turkey showed an average of 54.26% (Alege and Musapha, 2013). There was no significant difference in the oil content of Tamil Nadu sesame varieties in comparison to Turkish varieties. In another study, the oil content of 23 sesame samples from Nigeria were analyzed from 18 traditional and five developed accessions collected from 10 states in Nigeria. Among them, IBA II sesame variety from South West had high oil content of 58.85%. Nigerian varieties showed higher oil content than the Tamil Nadu varieties (Azeez and Morakinyo, 2014). The Nigerian sesame varieties were crossed for obtaining good oil, yield and fatty acid content, the highest oil content observed in S530 x PACH was only 57.58% (Mondal and Bhat, 2010). Genetic diversity based studies on Indian germplasm were used to estimate genetic differences among populations for seed yield and other characters (Bisht et al., 1998). However, there are no reports on oil content and quality parameters with genetic diversity on Indian germplasm. Genetic variation in Indian germplasm belonging to Rajasthan and North-eastern parts of India was observed through RAPD analysis to evaluate the genetic potential of Indian sesame varieties (Bhat et al., 1999). A RAPD study showed genetic distance coefficient of 0.35 among 58 accessions of sesame in Indian subcontinent and other countries (Bhat et al., 1999). The findings of this study would enable a breeder to link the genotype and oil parameters to the genetic cluster. Besides genetic factors, environmental factors and cultivation conditions may influence physiological and biochemical parameters (Bhat et al., 1999). Turkish sesame varieties showed variation coefficient ranging from 0.14 to 0.40 on RAPD analysis (Ercan et al., 2004). An ISSR based study on sesame germplasm from Korea and other countries showed minimum variation on polymorphism and distance coefficient of about 0.25 (Pham et al., 2011). Similarly, amplified fragment length polymorphism study also showed low genetic diversity of 0.14 to 0.21 among 32 sesame germplasm (Laurentin and Karlovsky, 2006). These suggest that the genetic diversity observed in several different germplasm collection are low hence other factors could play a role in oil content and yield.

Alpha linolenic acid content

Quantification of Alpha linolenic acid was performed in

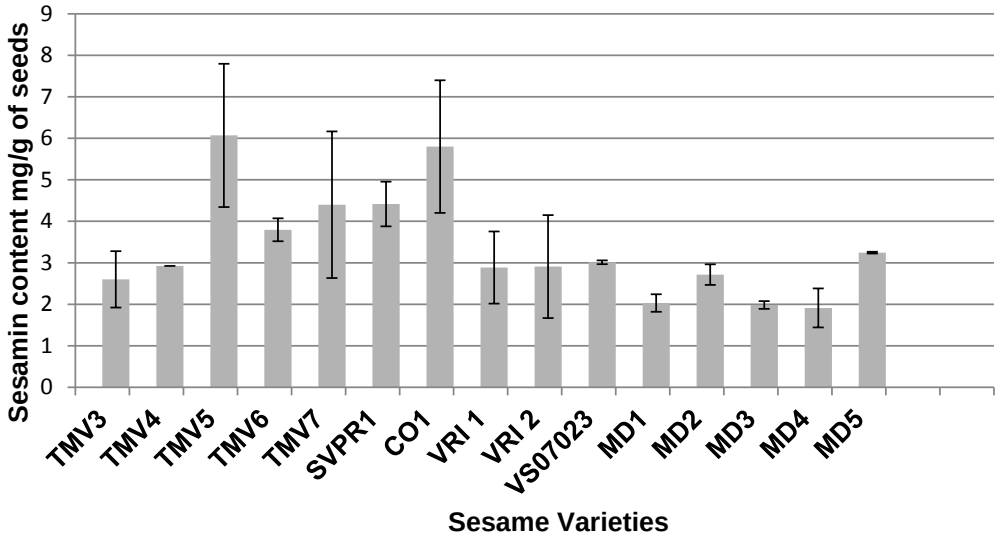

Figure 1. Sesamin content of *Sesamum indicum* L. germplasm collection of Tamil Nadu. Bars represent the amount of sesamin present in mg/g of seed. Error bars indicates mean ± SD, n=3 experiments.

Tamil Nadu sesame germplasm collection to evaluate the existing genotypes for improvement. Its quantity was very low in sesame accessions and ranged from 0.1 to 0.55 % in Tamil Nadu sesame seeds. The Maximum ALA content was found in TMV7 (0.55%) and the minimum ALA content was observed in VS07023 (0.1%) (Table 2). The AKT 101 variety (N 62 × N12-19) from Maharashtra had high ALA content of 1.17% (Mondal and Bhat, 2010). Sesame wild species like *S. mulayanum* (CAZRI, ABB-99-33)*, S. malabaricum* (IC 253971) and *S. alatum* (IC 253950) had no alpha linolenic acid (Mondal and Bhat, 2010). Domestication and selection might have enhanced ALA to a certain extent. The ALA content of sesame seeds from different countries like China, Egypt, India, Japan, Mexico, Myanmar, Thailand and Burkina Faso varies from 0.1 to 0.2% (Crew et al., 2006). The ALA content of Turkish varieties ranges from 0.53 to 0.60% (Unal and Yalcin, 2008). The Cambidi variety from Turkish had high level of ALA (0.60%). Worldwide studies indicate a wide variation in ALA content. A Chinese study on seed coat colour and ALA content reported no correlation between ALA content and seed colour (Philip, 2011).

Determination of sesamin content in sesame seeds

Sesaminis present in high levels compared to other lignans in sesame. The sesamin content of Tamil Nadu sesame varieties ranges from 2.03 to 6.45 mg/g of sesame seeds shown in Figure 1. The highest content of sesamin was found in TMV5 variety (6.45 mg/g of seeds). Similarly quantification of sesamin content was done in sesame seeds collected from different parts of North India and in commercial sesame oils. Sesame seeds from Assam show high sesamin content of 18.6 g/kg of oil. Low levels of sesamin content were found in Gujarat variety 3.1 g/kg of oil. Among commercial oils, AS brand (Agmark labeled oil) produced by crushing hulled sesame seeds had high level of sesamin content (9.0 g/kg).Similarly wide variation of sesamin content was seen in commercial oils of Assam and Gujarat sesame seeds (Hemalatha and Ghafoorunissa, 2004). These two states come under different geographical region with different soil and agro climatic conditions. Hence environmental factors could have a role on the sesamin content. However, Tamil Nadu germplasm grown in same geographical zone indicate higher difference suggesting a possible genetic contribution. Sesamin content in different colored of sesame seeds obtained from National Bureau of Plant Genetic Resources, New Delhi, India were analyzed. A white seeds variety, phuletil had high sesamin content of 38.43 g/kg whereas praghti another white seed variety showed the minimum level of sesamin 15.43 g/kg (Dar et al., 2014). Broad variation of sesamin content from 2.49 to 18.01 mg/g was observed in 215 sesame lines from core collection of China. They observed higher sesamin content in white sesame seeds than that of black, yellow, brown colours of sesame seeds (Wang et al., 2012). Sesamin content of black and white sesame seeds of Karnataka showed only slight variation of 3.031 and 4.663 g/kg of seed. Similarly commercially available sesame oils of Karnataka also showed slight variation in sesamin content of about 6.724 and 6.759 g/kg of sesame seed oil (Bhatnagar et al., 2013). In contrast, Tamil Nadu germplasm showed higher sesamin content in brown seeds (Figure 1). TMV5 and CO1 of Tamil Nadu sesame seeds recorded 6.45 and 5.9 mg/g of sesamin, respectively.

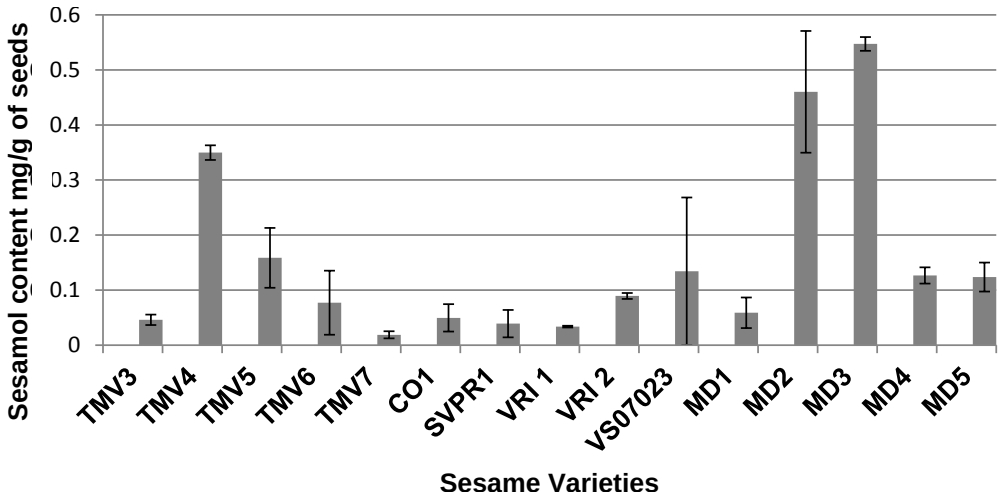

Figure 2. Sesamol content of *Sesamum indicum* L. germplasm collection of Tamil Nadu. Bars represent the amount of sesamol present in mg/g of seed .Error bars indicates mean ± SD, n=3 experiments.

Determination of sesamol content in sesame seeds

Sesamolin is another lignan present in sesame seeds. Sesamolin is hydrolysed during heating leading to the production of sesamol. Sesamol is the main reason for oxidative stability of sesame seed oil (Wan et al., 2014). Sesamol quantity in sesame varies between 0.03 and 0.55 mg/g of seeds in Tamil Nadu varieties and landraces (Figure 2). The highest sesamol content of 0.547 mg/g of seed was found in landrace MD3. The sesamol content of Burma black sesame seeds was less than 0.1 mg/g of seeds (Shyu and Hwang, 2001). The sesamol content of black sesame variety was 730 mg/kg and white sesame variety was found to be 68 mg/kg. The commercial expeller pressed sesame oils (CSO-A, CSO-B) showed variation of sesamol content ranging between 274 and 618 mg/kg of sesame oil (Bhatnagar et al., 2013). In Tamil Nadu accessions the sesamol content was high in white sesame seeds when compared to brown sesame seeds. Sesame oil produced by roasted sesame seeds at high temperatures before mechanical pressing can promote the flavor and colour of sesame oil (Singh et al., 2015). The roasted sesame seed oil has a higher sesamol content of 36 mg/kg of sesame seed when compared to unroasted which has only 7 mg/kg of seed (Wan et al., 2014). The sesamol content of unroasted sesame seeds was not detectable while those roasted at 180°C showed 235 mg/kg of sesame oil which further increased 10 fold when roasted at 250°C (Yoshida, 1994).

The total oil content of sesame germplasm of Tamil Nadu ranged between 50 and 56%. The alpha linolenic acid content of these sesame varieties was found to be 0.1 to 0.55% which is lesser than the Maharastra AKT101 which had 1.17% of ALA. Sesamin and Sesamol content were comparable to other germplasms from Turkey, China and North India. High sesamin content in Tamil Nadu varieties were discussed previously (6.45 mg/g) as they were close to *S. malabarium*. Quantification of these lignans would enable us to find the local varieties for further exploitation.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Abbreviations

ALA, Alpha linolenic acid; **mt,** metric tonnes; **ha,** hectare.

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Full Length Research Paper

Morphophysiology of peppermint irrigated with salt water and bovine biofertilizer

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Peppermint is a medicinal plant grown worldwide, but it has not been extensively studied, especially the use of saline water for its cultivation. In this sense, the objective of this study is to evaluate the effect of saline waters on peppermint cultivation under the application of bovine biofertilizer. The experiment was carried out from September 2015 to December 2015 in a greenhouse of the Center of Human and Agrarian Sciences of the State University of Paraíba (UEPB) in the municipality of Catolé do Rocha-PB, Brazil. The experimental design was completely randomized, in a factorial scheme of 5 × 2, with 8 replicates. The treatments consisted of electric conductivity combined with irrigation water (ECw) of 1.0, 2.0, 3.0, 4.0, and 5.0 dS m^{-1} in the presence and absence of bovine biofertilizer. The increase in electrical conductivity of the irrigation water levels from 1 dS m^{-1} reduced the growth, development and production of peppermint biomass. Peppermint plants that received bovine biofertilizer had superior results in growth and biomass production. The application of bovine biofertilizer attenuates the effects of salty peppermint. The growth and production of peppermint biomass increased when the plants are irrigated with low salinity water (1 dS m^{-1}) using bovine biofertilizer.

Key words: *Mentha piperita* L., electrical conductivity, mitigating the salt stress.

INTRODUCTION

Peppermint *(Mentha piperita* L.) belongs to the Lamiaceae family. Originating in Europe and North Africa, it is widely cultivated in Brazil. It is a hybrid coming from the crossing of *Mentha aquatica* L. and *Mentha viridis* L. Botanical synonyms: *Mentha citrata* Ehrh. It is also known as peppermint, mint, mint-kitchens, and sandalwood. It is an annual or perennial herb, aromatic, about 50 cm high, erect, and with branches that are dark green to purple. It has a quadrangular structure. The leaves have opposite arrangememt, elliptic-acuminate,

jagged and pubescent. It has lilac flowers which are gathered in axillary glomeruli (Grandi, 2014).

The impact of salinity is considered a limiting factor in agricultural production in the arid and semi-arid regions of the world. In order to reduce the negative impacts on agriculture, alternatives are sought for the reutilization of unused areas, such as agricultural varieties tolerant to these conditions, as well as the prospection of substances capable of reversing the damage caused by salinity during cultivation (Kaiser et al., 2016).

To attenuate the effects of saline stress, organic input has been recommended as the bovine biofertilizer. Several studies have demonstrated the positive effects of organic inputs (El-Dardiry, 2007; Miranda et al., 2011). Among them is bovine biofertilizer; it improves the soil in terms of aeration (Aidyn et al., 2012) and it has complex substances used to mitigate the depressive effects of water salinity on plants.

In addition, bovine biofertilizer has a positive action; its composition has many beneficial substances. Among them are the humic substances which promote the reduction of the osmotic potential of soil solution, and stimulate the absorption of water and nutrients by plants in saline environments. The application of biofertilizer in the soil can induce increased osmotic adjustment in the plants by the accumulation of organic solutes, promoting the absorption of water and nutrients in adverse saline environments (Baldotto and Baldotto, 2014).

Due to the lack of studies on peppermint, especially in the semi-arid region, where it has low rainfall indexes, the use of low quality water in irrigation becomes relevant in this sense, to evaluate the effect of saline waters in the cultivation of mint using bovine biofertilizer.

MATERIALS AND METHODS

The experiment was carried out from September 2015 to December 2015 in a greenhouse at the Center for Human and Agrarian Sciences of the State University of Paraíba (UEPB) in the municipality of Catolé do Rocha-PB, (6° 20'38 "S ; 37° 44'48 "W). The climate of the municipality, according to the Koppen classification, is of the type BSW', that is to say, warm and dry type of steppe, with average monthly temperature superior to 18°C, throughout the year and 275 m of altitude.

Mint seedlings grown in 128-cell expanded polystyrene trays containing commercial Plantmax Hortaliças HT® substrate were obtained. The seedlings were grown in a greenhouse, with 60% shading, for 15 days, until it reaches about 10 cm in height. After acclimatization, they were transplanted to polyethylene pots with capacity of 8 dm³ in September 2015.

The experimental design was completely randomized, in a factorial scheme of 5 × 2, with 8 replications. The treatments consisted of electric conductivity combined with irrigation water (ECw) of 1.0, 2.0, 3.0, 4.0 and 5.0 dS m^{-1} in the presence and absence of biofertilizer bovine. The experimental units consisted of three plants.

To fill the vessels, a flavic Neosol was used with a sandy loam clay texture. Samples were collected in 0 to 20 cm layer in a native area located on UEPB campus. A subsample was withdrawn andanalyzed chemically, with the following characteristics (Table 1).

Bovine biofertilizer was obtained by anaerobic fermentation, that is, in a hermetically sealed environment. To release the methane gas at the top of each biodigester one end of a thin hose was coupled and the other end was immersed in a vessel with water. For the preparation of the biofertilizer, 70 kg of bovine manure from lactating cows and 120 L of water were used. 5 kg of sugar and 5 L of milk were added to accelerate the metabolism of the bacteria.

Biofertilizer treatments were applied 15 days after sowing (DAS), at 8 days interval; totalling 6 applications of 10% dosage of the vessel volume (0.8 dm³). Prior to application, bovine biofertilizer was diluted in water (5%), after which it was subjected to screen filtration to reduce the risks of obstruction of the watering system. The biofertilizer was analyzed (Table 1).

The water used for the irrigation was supplied from the local water supply and had electrical conductivity of 1.0 dS m^{-1}. Treatments with salinities were performed at 15 days after emergence. The plants were irrigated daily with each type of water, starting on the fifteenth day after sowing. Irrigation was performed manually by watering, providing a blade sufficient to raise the soil moisture at the field capacity level.

Different ECws were obtained by the addition of sodium chloride (NaCl) to the water from the local supply system, according to Rhoades et al. (2000). The quantity of salts (Q) was determined by the equation:

$$Q\ (mg/L^{-1}) = ECw \times 640$$

where ECw (1.0 dS m^{-1}) is the desired value of electric conductivity from water.

The height of the plant, stem diameter and leaf area were evaluated at 30 and 90 DAS. In the measurement of plant height, a measuring tape graduated in cm was used, measuring the distance between the collar and the apex of the plant (Insertion of the youngest fully formed leaf). Measurements of stem diameter were performed with a digital caliper at 2 cm above the neck of the plant. The leaf area was obtained by measuring the width and length of the leaf.

From the mean monthly values of plant height, stem diameter and leaf area, their respective absolute growth rates (AGR) and relative growth rates (RGR) were calculated according to Benincasa (2003).

At 90 days after sowing, harvesting was performed in which the plants were separated into roots, stem and leaf; they were dried in an oven with forced ventilation at 65°C until constant weight was obtained. The fresh mass of the aerial part was determined by weighing in a precision scale of 0.0001 g. Later the parts of the plants (root, stem and leaves) were weighed in a precision balance of 0.0001 g. The total dry matter production data were used to calculate the percentages partitioned between vegetative organs and the rate of salinity tolerance. The data from saline treatments with the control (ECw = 1.0 dS m^{-1}) were compared, according to the methodology of Aquino et al. (2007).

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Table 1. Soil chemical attributes and bovine biofertilizer used in the experiment. Catolé do Rocha - PB, UEPB, 2015.

Characteristics	Soil	Bovine biofertilizer
$CaCl_2$ pH	5.02	4.68
EC (dS m^{-1})	0.60	4,70
Ca^{+2} (cmol $_c$ dm^{-3})	4.63	3.75
Mg^{+2} (cmol $_c$ dm^{-3})	2.39	3.30
Na^{+} (cmol $_c$ dm^{-3})	0.30	1.14
K^{+} (cmol $_c$ dm^{-3})	0.76	0.71
P (mg dm_3)	0.70	14,45
Al^{3+} (cmol $_c$ dm^{-3})	0.00	0.00
H^{+} + Al^{3+} (cmol $_c$ dm^{-3})	1.00	1.00
SB	7.78	7.76
Organic matter (g kg^{-1})	8.05	8.00

The data obtained were evaluated by analysis of variance using F test at 0.05 and 0.01 probability level. For significance level, linear and quadratic polynomial regression analysis was performed using the statistical software SISVAR 5.0. (Ferreira, 2011).

RESULTS AND DISCUSSION

There is interaction between the electrical conductivities of the irrigation water (ECw) and the bovine biofertilizer applied on the plant height (PH) at 30 and 60 DAS and for stem diameter (SD) at 90 and 120 DAS. The factors, electrical conductivity in irrigation water and the application of bovine biofertilizer exerted significant effects on all variables analyzed in the periods evaluated.

The plants irrigated with saline water (5.0 dS m^{-1}) showed a reduction in plant height in all periods. However, those treated with bovine biofertilizer demonstrated superiority in values, with 2 reductions, 22 cm at 30 DAS (Figure 1A) and 1.38 cm at 60 DAS (Figure 1B); with those without bovine biofertilizer had reductions of 1.19 cm at 30 DAS (Figure 1A) and 2.24 cm at 90 DAS (Figure 1B), with increased ECw.

At 90 and 120 DAS, plant height as a function of ECw decreased from 1.99 cm to 90 DAS (Figure 1C) and from 2.03 cm to 120 DAS (Figure 1C) for each unit increase of ECw. Bovine biofertilizer positively influenced plant height at 90 and 120 DAS (Figure 1D); the plants that received bovine fertilizer obtained values of 24.32 and 26.1 cm at 90 and 120 DAS, respectively. In addition, in the periods evaluated, bovine biofertilizer provided higher values, demonstrating the beneficial effect of the organic input on the height of the peppermint plant.

Bione et al. (2014) studied basil culture (*Ocimum basilicum* L.) and found that the electrical conductivity in the irrigation water reduced 0.0117 m plant height with ECw unit 49 days after transplantation (DAT). In tomato, Guedes et al. (2015) observed that saline water negatively affected plants irrigated with water of 3.5 dS m^{-1}; there was a linear reduction from electrical conductivity.

Bovine biofertilizer has humic substances that improve soil structure, increase cell division and permeability of cell membranes; and consequently, provide greater absorption of water and nutrients in plants subjected to saline stress, leading to greater growth (Khaled and Fawy, 2011).

It was observed that increased ECw promoted a reduction in stem diameter at 30 DAS in the order of 0.36 mm and at 60 DAS of 0.37 (Figure 2A). However, at 60 DAS, bovine biofertilizer positively influenced the diameter of the peppermint stem, presenting a value of 2.06 mm, while without bovine fertilizer application, a lower value (1.95 mm) was observed (Figure 2B). At 90 and 120 DAS, the peppermint plants reduced with increased Ecw. With increased Ecw, there was a decrease in the order of 0.28 mm in the presence of biofertilizer and 0.39 mm without bovine fertilizer at 90 DAS (Figure 2C); there was reduction of 0.26 mm with bovine biofertilizer and 0.39 mm without bovine fertilizer (Figure 2D).

In the culture of eggplant and castor bean, Lima et al. (2015, 2008) observed that increased saline in the irrigation water promoted a reduction in stem diameter. Studying tomato, Medeiros et al. (2014) found that the interaction of salinity × biofertilizers has significant effect and that the plants that received bovine biofertilizer had superior results with maximum diameter value at maximum salinity estimated to be 2.64 irrigation water dS m^{-1}.

Reduction in the diameter of the stem occurred due to the toxic effect of the ions Na^{+} and Cl^{-} which cause

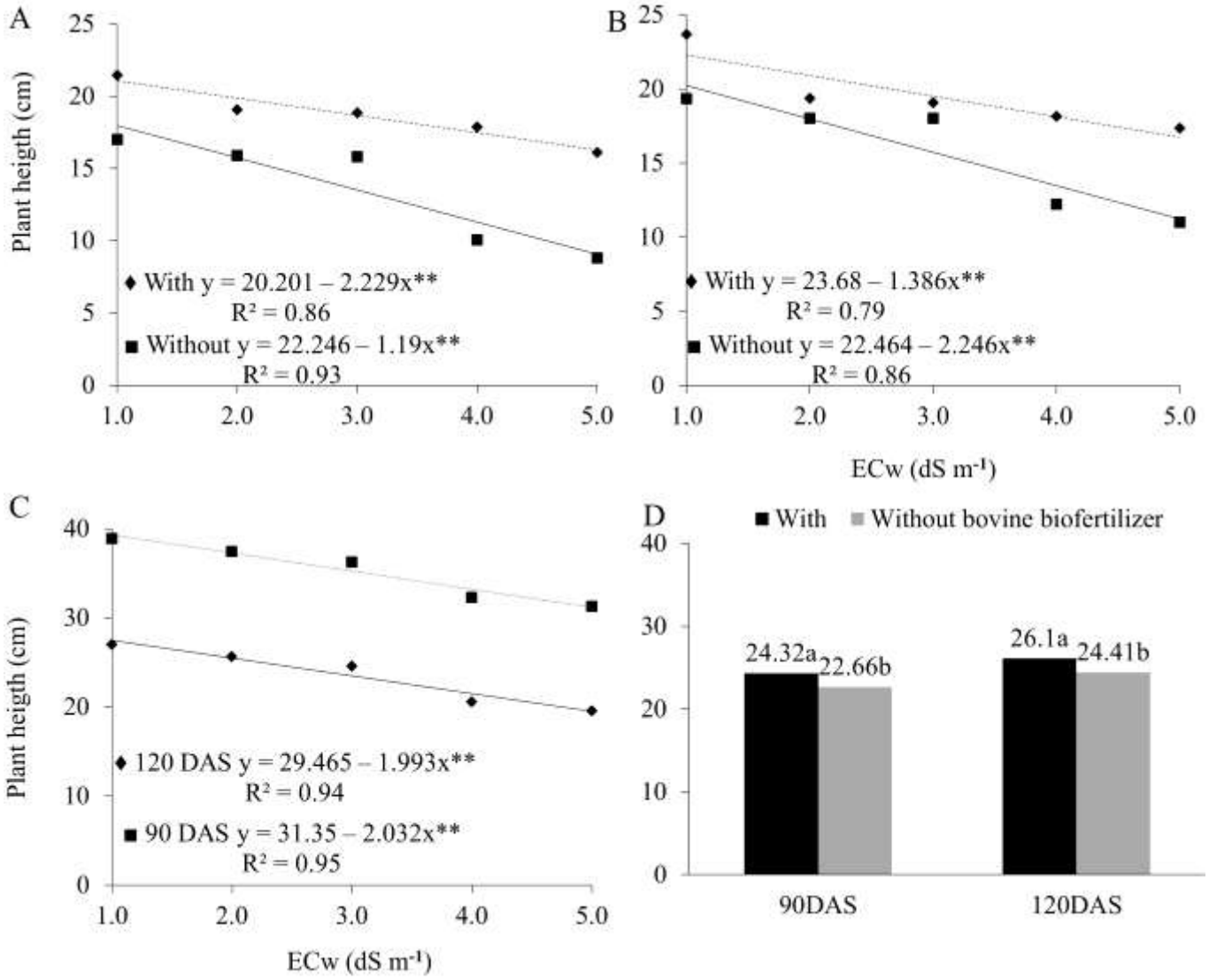

Figure 1. Effect of electrical conductivity in the irrigation water over the height of the ground peppermint plant with and without biofertilizer to 30 (A), 60 (B), and 90 to 120 DAS (C and D).

decreased absorption of water and nutrients and imbalance in the cationic sheet and plant metabolism. This leads to loss in growth and production (Nivas et al., 2011). However, with the application of bovine biofertilizer, plants irrigated with saline water were superior, because of the organic raw material and nitrogen supply. Thus, it is believed that bovine biofertilizer reduces the effect of high concentrations of saline waters and favors the development of plants (Sá et al., 2015).

For the leaf area, there are data that decrease the linear model in all periods (Figure 3A); this shows the extent to which increased ECw of 1.0 to 5.0 dS m^{-1} led to reductions of 156.66, 157.06, 85.12 and 83.76 cm^2 for the leaf area of peppermint at 30, 60, 90 and 120 DAS, respectively (Figure 3A). However, the plants that received bovine biofertilizer presented a larger leaf area and higher results in the periods evaluated: 580.55, 634.26, 852.55, and 898.51 cm^2 at 30, 60, 90 and 120 DAS, respectively against 502.2, 55.42, 788.7, and 827.95 cm^2 at 30, 60, 90 and 120 DAS, respectively (Figure 3B).

The decline in leaf area, emergence of new leaves and death and leaf fall occur due to the effects of saline stress, since the plant uses these strategies as a way to reduce water losses (Mahmoud and Mohamed, 2008). The emergence of new leaves and/or foliar senescence occur also because these organs are

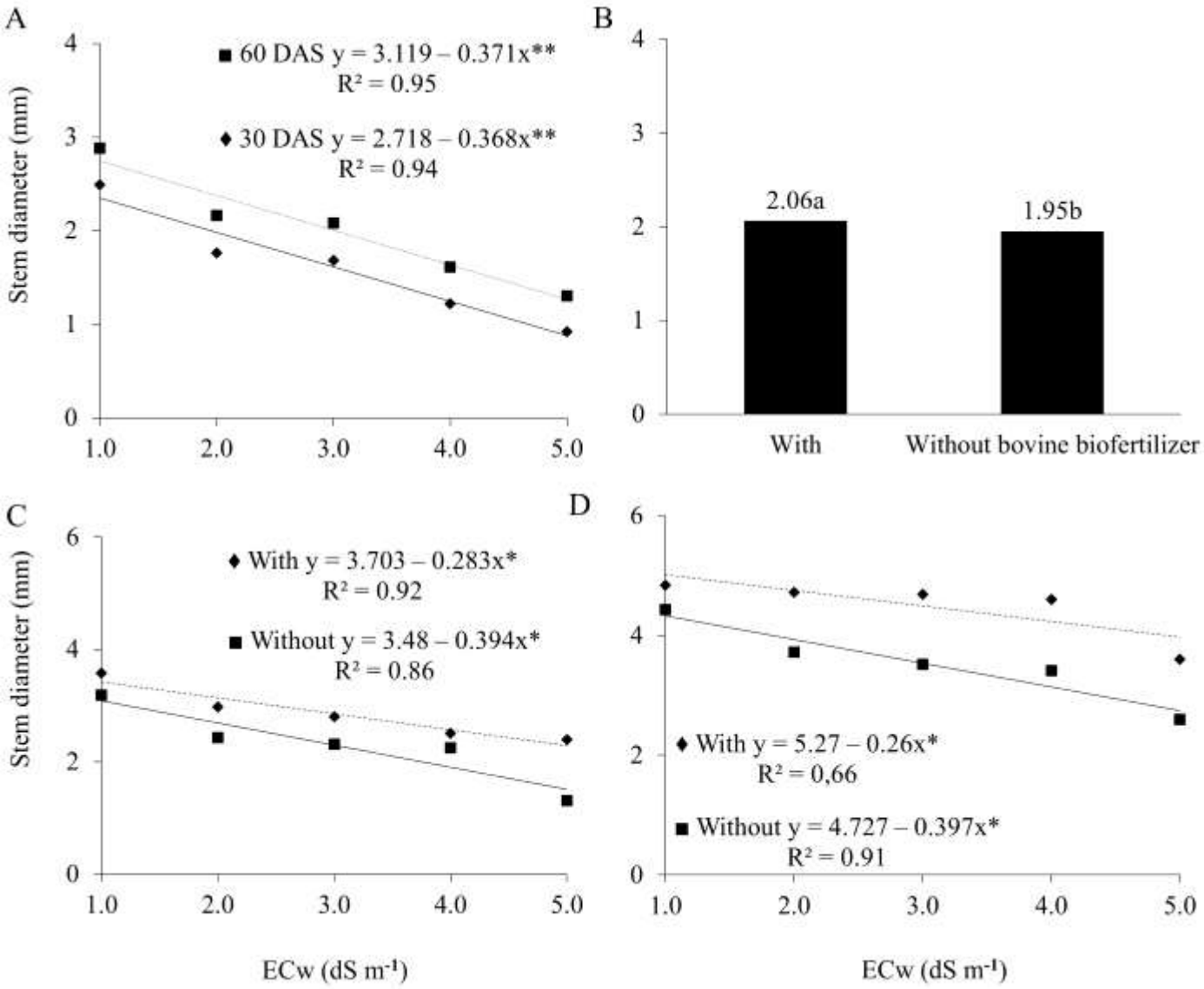

Figure 2. Effect of electrical conductivity in the irrigation water on the diameter of peppermint stem at 30 and 60 DAS (A) in the soil with and without biofertilizer to 60 (B), 90 (C) and 120 DAS (D).

sensitive to salinity and reduce in size and number in the presence of high concentrations of salts. The deleterious effect of salinity on leaf area was also observed by Medeiros et al. (2011), Vieira et al. (2016) and Lycoskoufis et al. (2012) in tomato.

In addition to supplying nutrients and organic matter to the plants, the application of bovine biofertilizer contributes to the tolerance of the plants to salinity, providing improvements in the germination, growth and biomass production (Lancet et al., 2006).

There is a significant effect of electrical conductivities on irrigation water (ECw) in all variables analyzed. For the bovine biofertilizer factor, only significant effect was observed for the absolute growth rate in plant height (AGRph), absolute growth rate (AGRsd) and relative stem diameter (RGRsd), dry mass of the root (DMR), dry mass of the aerial part (DMAP) and total dry matter (TDM). There was a significant effect of the ECw × bovine biofertilizer interaction on the variables AGRph, RGRph, AGRsd, RGRsd and DMR.

It was observed that the interaction between ECw and bovine biofertilizer exerted significant effects on absolute (AGRph) and relative (RGRph) growth rates of plant height and absolute (AGRsd) and relative (RGRsd) stem diameter; with increased ECW, there were reductions of 0.0083 cm^{-1} day in AGRph (Figure 4A) and 0.0062 cm

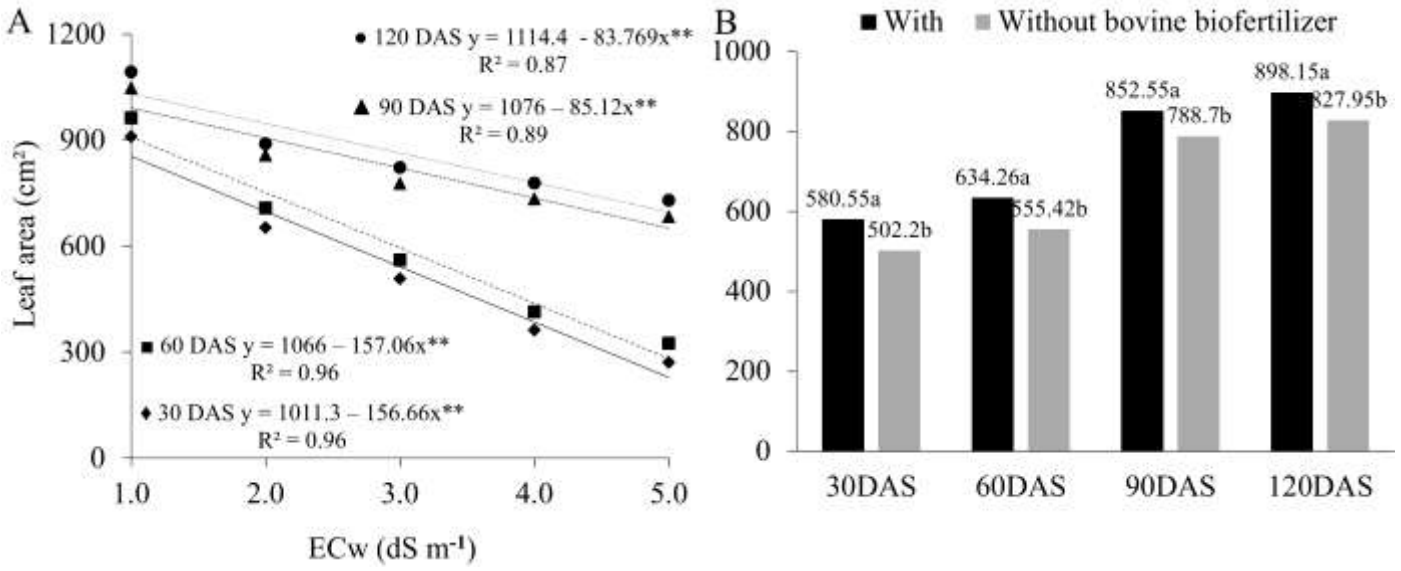

Figure 3. Effect of electrical conductivity in the irrigation water (A) on the leaf area peppermint soil with and without biofertilizer (B) 30, 60, 90 and 120 DAS.

cm^{-1} day^{-1} in RGRph (Figure 4B) in plants treated with biofertilizer against 0.0124 cm^{-1} in day AGRph (Figure 4A) and 0.0106 cm^{-1} day^{-1} in RGRph (Figure 4B) in plants treated with bovine fertilizer.

In the absolute growth rate of leaf area (AGRlf), it is noticed that ECw interfered negatively, decreasing linearly with reductions of 0.0088 cm^2 day^{-1} for each unit increase in ECw (Figure 4C). It can be seen in Figure 4D that the peppermint plants when subjected to ECw 5.0 dS m^{-1} showed relative growth rate of leaf area (RGRlf) lower compared to plants irrigated with water of lower salinity (1.0 dS m^{-1}), presenting reductions 0.0037 cm^2 cm^2 day^{-1} per unit increase of ECw.

The peppermint plants had reduced absolute growth rate of stem diameter (AGRsd) of 0.0023 mm day^{-1} when the plants were treated with bovine biofertilizer and 0.0025 mm day^{-1} without bovine biofertilizer (Figure 4E); while, the relative growth rate of stem diameter reduction was 0.002 mm^{-1} day with biofertilizer bovine mm and 0.0021 mm^{-1} day^{-1} without bovine biofertilizer (Figure 4F).

The reduction in plant growth when subjected to salinity is caused by water deficit caused by excess soluble salts in the root zone. It causes a decline in turgescence, consequently resulting in a decrease in cell expansion, reducing the growth rate of plants (Khalid and Silva, 2010). This occurs due to the closure of the stomata and consequently less CO_2 assimilation limiting the photosynthetic processes (Debez et al, 2008; Taarit et al., 2010). It can also be caused by the energy expenditure that is required in the synthesis of organic solutes and in the processes of compartmentalization and regulation of ion transport (Mendonça et al., 2007).

Plant growth rate is one of the most important parameters for evaluating the effects of saline stress as well as the capacity of the plant to overcome salinity, since plant growth processes are particularly sensitive to the effect of salts (Morais et al., 2011). Reductions in growth rates are mainly due to the deleterious effect of excess salts on plant metabolism (Santos et al., 2013).

ECw had a negative influence on shoot fresh mass (DMAP), dry shoot mass (DSM), dry mass of the root (DMR) and total (DMT) of the peppermint plants, according to the regression equations. The model to which the data fit better was linear, so that as the ECw increased, there were decreases of 2.62 and 3.25. The highest gains in DMAP, MSPA, DMR and DMT were observed in the plants treated with bovine biofertilizer, with values of 40.85, 0.89 and 4.20 g in DMAP, MSPA, DMR and DMT, respectively (Figure 5A); 11.05, 2.74 and 1528 g, respectively (Figure 5B).

When the plants are subjected to saline environments, some things occur, such as reduction in biomass production, which has already been observed by several authors (Freire et al., 2010; Gomes et al., 2011; Medeiros et al., 2011). Thus, to reduce energy costs, plants reduce leaf area, among other mechanisms to reduce water losses; as a consequence, there is less accumulation of biomass, since there is a proportional relationship between transpiration and plant production (DIAS et al., 2011).

In addition, saline stress has negative effects on the plant, such as changes in root growth and development; consequently, it interferes with the absorption of ionic

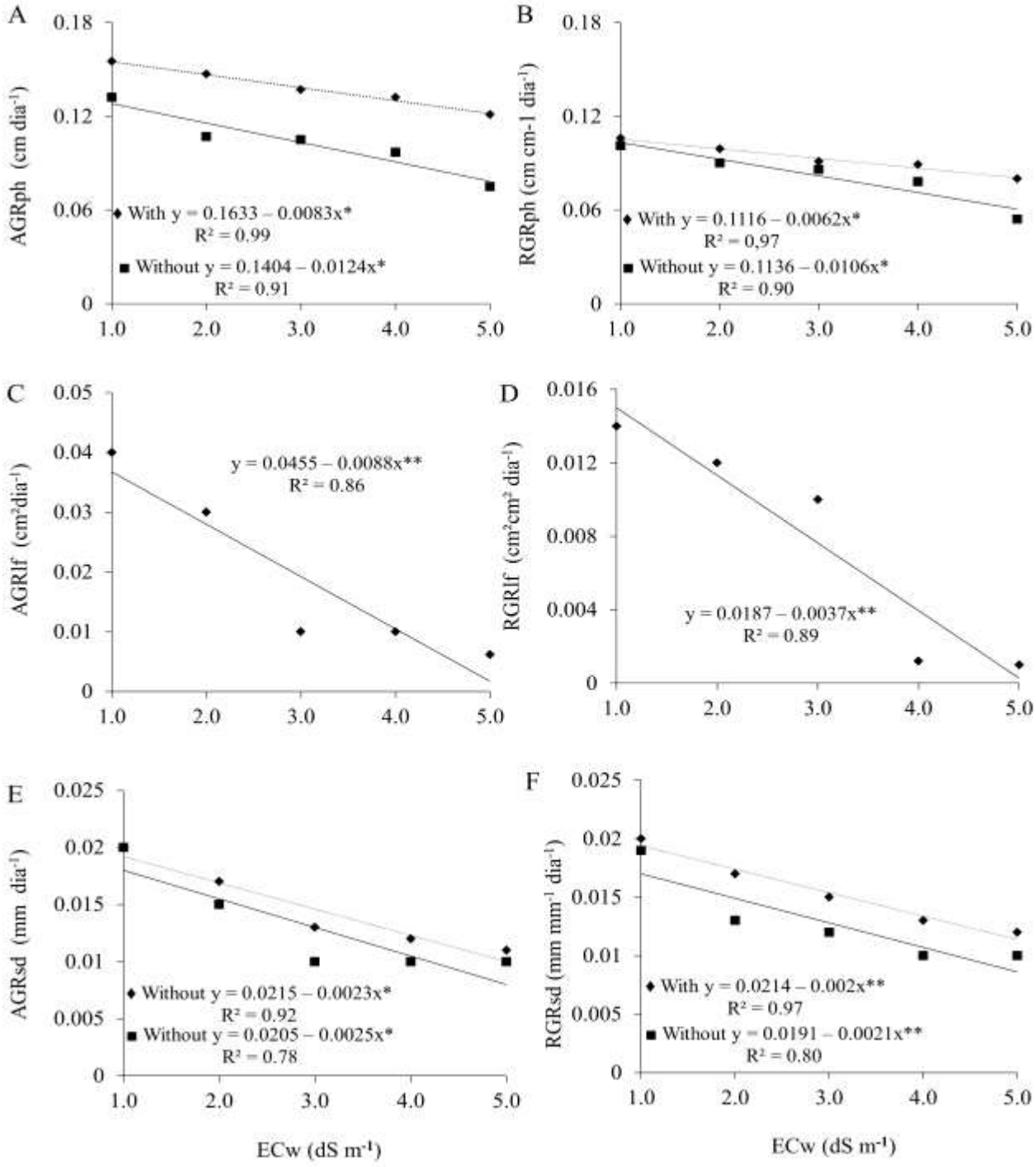

Figure 4. Effect of electrical conductivity in the irrigation water on the absolute growth rates and relative plant height (AGRph (A), RGRph (B)), leaf area (AGRlf (C), RGRlf (D)) and of stem diameter (AGRsd (E), RGRsd (F)) in the soil with and without peppermint biofertilizer.

water, hindering the development of crops; also, the well developed root system provides a greater area of absorption (Soares et al., 2011). However, it is not possible to determine the effect of the plant's nutrients on the nutrient content of the plant.

Tabatabaei et al. (2007), studying peppermint (*M. piperita* L.) grown in hydroponics, found that increasing concentrations of electrical conductivity solutions

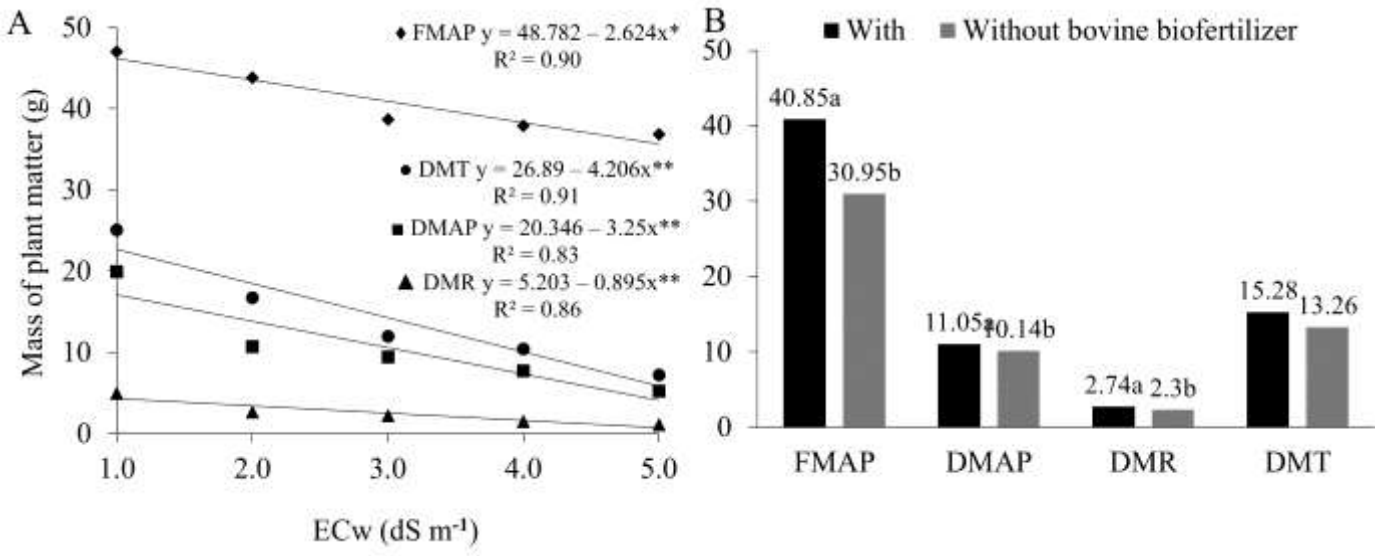

Figure 5. Effect of electrical conductivity in the irrigation water (A) on the mass of the plant matter on the ground with and without biofertilizer (B) peppermint.

adversely affected growth, essential oil content and production of biomass. The same authors found that the highest and lowest accumulation of fresh weight of the plants were obtained in 1.4 and 5.6 dS m^{-1}, respectively. Aziz et al. (2009) found that the mint biomass was reduced when plants were subjected to salinity of 2.56 dS m^{-1}, leading to a reduction of 30%. Tabatabaie and Nazari (2007) also observed that the concentration of NaCl adversely affect the growth of spearmint, wherein the higher biomass yields occurred at moderate levels of electrical conductivity (1.4 to 2.8 dS m^{-1}).

Heidari (2012), working with basil (*O. basilicum* L.) subjected to salinity in nutrient solution, observed a reduction of 17.8% in the mass production of fresh basil when the plants were irrigated with salinity waters over non-saline condition. The reduction in the biomass of the aerial part occurs due to the deviation of energy as a result of the increase of the salinity levels of the soil (Garcia et al., 2007).

In order to reduce the effects of salinity, bovine biofertilizer was used, since it has some beneficial elements, mainly the diversity and availability of the essential nutrients used for biological activity (Alves et al., 2009). In addition, it has many beneficial substances: the humic substances which promote the reduction of the osmotic potential of soil solution, and stimulate the absorption of water and nutrients by the plants, in saline environments (Aydin et al., 2012).

In cherry tomato crop, Medeiros et al. (2011) observed that the application of bovine manure biofertilizers (with and without the addition of molasses, milk and agricultural gypsum) provided a positive effect on biomass production even with increased salinity of irrigation water.

Behavior similar to the previous variables can be observed in the shoot root ratio and tolerance index, where the data conformed to the linear decreasing model, with a reduction of 0.065 in the shoot root ratio (Figure 6A) and 16.55% in the tolerance index (Figure 6B). In each unit increase of ECw, in higher salinity (5.0 dS m^{-1}), 0.52 value was obtained in the relation of root and shoot ratio plus 28.75% tolerance index; when the plants were irrigated with low salinity water (1.0 dS m^{-1}) a maximum value of 0.79 was obtained in the root and shoot ratio plus 100% of tolerance index.

Studying mint (*O. basilicum* L.), Bernstein et al. (2010) did not find a significant effect on the root/shoot ratio of the hydroponic basil, as a function of salinity. While Bione et al. (2014) observed that the ratio R/PA increased significantly with increasing salinity (8.94% per dS m^{-1}).

The tolerance of peppermint to salinity has been studied by several researchers, such as Khorasaninejad et al. (2010) who verified that the culture is considered moderately tolerant to salinity. However, the plants respond differently to salinity, that is, there is great variability depending on the species, genotype, the phenological stage of the same genotype and salt exposure period (Parida and Das, 2005; Munns and Tester, 2008).

Melo Filho et al. (2016) in peanut crop, Véras et al. (2016) in castor bean and Alves et al. (2016) in cotton observed that bovine biofertilizer does not mitigate the effect of salt stress; however, better results in growth rate, production and tolerance can be observed with the application of this input.

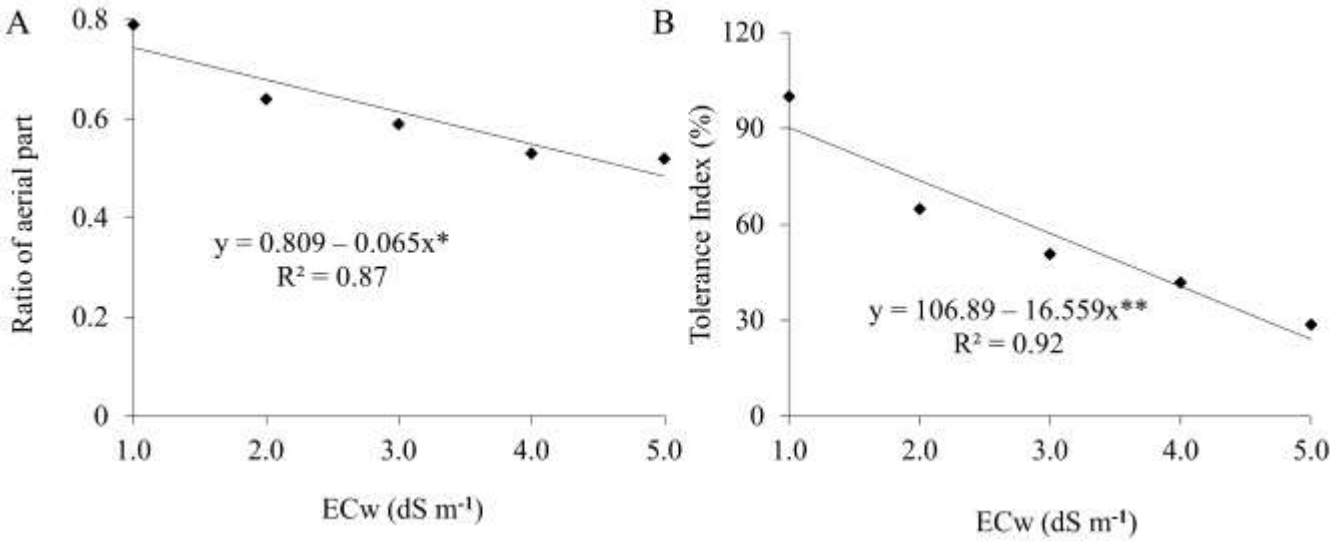

Figure 6. Effect of electrical conductivity in the irrigation water over the root shoot ratio (A) and tolerance index (B) peppermint.

Conclusion

The increase in electrical conductivity of the irrigation water levels from 1 dS m^{-1} reduced the growth, development and production of peppermint biomass. Peppermint plants that received bovine biofertilizer showed superior results expressed in growth and biomass production. The application of bovine biofertilizer attenuates the effects of salty peppermint. The growth and production of peppermint biomass increase when the plants are irrigated with low salinity water (1 dS m^{-1}) in the presence of bovine biofertilizer.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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