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Full Length Research Paper

Ripening of sapodilla fruits (*Manilkara zapota* [L.] P. Royen) treated with 1-methylcyclopropene after refrigeration

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An experiment was conducted during the year 2010 to evaluate the effect of the treatment with 1-methylcyclopropene (1-MCP) on the ripening process of sapodilla fruits, after refrigeration. The fruits were exposed to 0 and 1 $\mu\text{L/L}$ of 1-MCP for 24 h in airtight chambers at 25°C, after which they were stored at 16°C for four periods of refrigeration (0, 11, 18 and 25 days). At the end of each refrigeration period, the samples were allowed to ripen at 25°C. The results demonstrated the effectiveness of 1-MCP in significantly delaying ($P \leq 0.05$) the ripening process of sapodilla fruits and extending the post-harvest life to 28 days. It was possible to observe a reduction and delay in the climacteric maximums of ethylene and respiration, and a delay in the maximum activity of the pectin methyl esterase enzyme (PME), while weight loss was also reduced. In general, the quality characteristics of ripe fruits were maintained, except for a significant increase in the total soluble solids content of the fruits treated. These results indicate that the treatment with 1-MCP, combined with refrigeration, is an adequate alternative for increasing the shelf life of sapodilla with the aim of facilitating its commercialization.

Key words: Sapodilla, 1-methylcyclopropene, ripening, post-harvest, shelf life, refrigeration.

INTRODUCTION

The sapodilla (*Manilkara zapota* (L.) P. Royen) is a fruit species native to the tropical Americas which is said to have originated from southern Mexico. The fruit is sweet with a pleasant aroma and is greatly appreciated by a significantly large group of consumers (Balerdi and Shaw, 1998; Ma et al., 2003). India is the main producer of

sapodilla, with a production of 1,346,000 tons (Indian Horticulture Database, 2010). The production of this fruit in Mexico is estimated at 20,000 tons, mainly from the states of Campeche, Yucatán and Veracruz (SAGARPA, 2011).

Physiologically, sapodilla fruits exhibit a climacteric

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behavior in postharvest (Lakshminarayana, 1979); ripening occurs rapidly and is characterized by a significant increase in the respiration rate and ethylene production, all of which classify it as a highly perishable fruit with a short shelf life, making its commercialization more difficult. Depending on the variety and the agro-climatic conditions of production, the fruit ripens at 26°C between 8 and 10 days after harvesting (Morais et al., 2006). Any increase in the shelf-life of this product, therefore, would contribute to an improvement in its commercialization.

The fact that the sapodilla is highly perishable due to its climacteric nature, has made it necessary to study technologies of postharvest management that prolong its shelf life and favor its commercialization in more distant markets (Ganjyal et al., 2003). Ethylene action inhibitors, 1-methylcyclopropene (1-MCP) has been found to delay the ripening process and the onset of senescence in fruits. In their study, Sisler and Serek (1997) reported that 1-MCP, when used at very low concentrations (0.0025-1.0 $\mu\text{L/L}$), blocks the ethylene receptors, preventing the physiological action of this phytohormone on plant tissue. It has been demonstrated that this compound influences the physiological responses of the fruit during ripening, which include ethylene production, respiratory intensity, tissue softening, weight loss and degradation of the cell wall (Blankenship and Dole, 2003; Watkins, 2006). The activity of 1-MCP has been studied in a wide range of fruits with the objective of delaying the ripening process, prolonging shelf life and maintaining quality; for example, in apples (Kashimura et al., 2010), papaya (Manenoi et al., 2007), pears (Calvo and Sozzi, 2009), plums (Luo et al., 2009) and mangosteen (Piriavinit et al., 2011).

As a plausible alternative for prolonging the shelf-life of sapodilla, this study evaluated the effect on the ripening process of fruits treated with 1-MCP following a period of refrigeration.

MATERIALS AND METHODS

Fruit procurement

Five hundred and forty (540) sapodilla fruits were harvested in November, 2010 from an orchard located in the municipality of Cansahcab, Yucatan. The quality of fruits was selected based on the stage of physiological maturity. Maturity was determined by the absence of latex (Sulladmath and Reddy, 1990) and a less grainy texture of the peel (Araújo et al., 2001). At the time of harvest, the fruits were averaged of the following characteristics: average weight 200 g, a length of 10 cm, firmness 196 N and acidity of 0.27 g of malic acid/100 g of fresh pulp. The samples were then transported to the laboratory for analyses.

Treatment of fruits with 1-MCP

135 sapodilla fruits were placed into airtight chambers (0.07 m^3) and exposed to 1 $\mu\text{L/L}$ of 1-MCP (SmartFresh, Rohm and Haas, USA) for 24 h at 25°C. Moreover, 135 fruits were placed in the

similar airtight chambers with the same temperature and treatment time conditions but without the 1-MCP treatment (0 $\mu\text{L/L}$ of 1-MCP for 24 h at 25°C). The respective dose was calculated based on product weight and container volume, considering that 1.6 g of powder releases 1.0 $\mu\text{L/L}$ of 1-MCP in 1.0 m^3 . The compound, previously weighed, was dissolved in a flask with 25 mL of distilled water at 40°C (Akbulak et al., 2009) and the mixture was shaken until the powder had dissolved completely. Subsequently, each flask was placed inside each airtight chamber containing the fruit to release the vapour of 1-MCP. The period between harvesting and the initiation of the treatments was two days.

After the treatment, a group of untreated fruit and fruit treated with 1-MCP were stored at 16°C (with refrigeration) for 11, 18 and 25 days, while the remaining fruit were kept at 25°C for ripening (without refrigeration). After each period of refrigeration, fruit were removed from storage and kept at 25°C until the ripening process was complete. The experiment was conducted with two replications. During the ripening period at 25°C, respiration rate, ethylene production, percentage of ripe fruits, percentage of weight loss and firmness were determined daily to reach the ripening of sapodilla, while the activity of the pectin methyl esterase enzyme (PME) was measured every second day.

When the fruits reached ripeness-consumption stage (this being characterized by the time the fruits were soft to the touch), a group of untreated fruit and fruit treated with 1-MCP were removed in order to measure titratable acidity, total soluble solids, reducing sugars, color and luminosity of the pulp.

Ethylene production and respiration rate

Respiration and ethylene production were measured daily using the same fruits of each treatment. Three fruits from each replication were sealed for 2 h at 25°C in 2 L plastic containers prior to gas sampling. A 2 mL gas sample was withdrawn by a syringe through a rubber septum and analyzed by a gas chromatograph (Varian Star model 3400, Walnut Creek, CA, USA). Carbon dioxide and ethylene were determined using a thermal conductivity detector (TCD) and flame ionization detector (FID) respectively, with a Porapak Q column. Injector and detector temperatures were both set at 250°C, and an isothermal program was run at 30°C. Helium was used as the carrier gas at a flow rate of 1 mL/min. Based on areas of standard gasses, concentrations of carbon dioxide and ethylene were calculated. Respiration rate was expressed as mL/kg/h while ethylene production rate was expressed as $\mu\text{L/kg/h}$.

Ripening and physico-chemical characteristics

The determination of ripeness for consumption was based on changes in firmness (softening) and was characterized as the moment at which the fruit was soft to the touch (Télez et al., 2009). The result was expressed in percentage of ripe fruits.

The accumulated weight losses were measured in percentage with respect to the initial weight of the fruits; ten individually weighed fruits were used each day after treatment. The measurement was made with a digital balance (OHAUS, Adventure Pro AV3102, USA) and results were expressed as percentage.

For the determination of soluble solids content (SSC, °Brix), titratable acidity (TA) and reducing sugar, five ripe fruits per replication were homogenized and the homogenates filtered through a cheese cloth to obtain clear juice. SSC was determined by a digital refractometer (Model PR-1, Atago, Tokyo, Japan) and expressed as

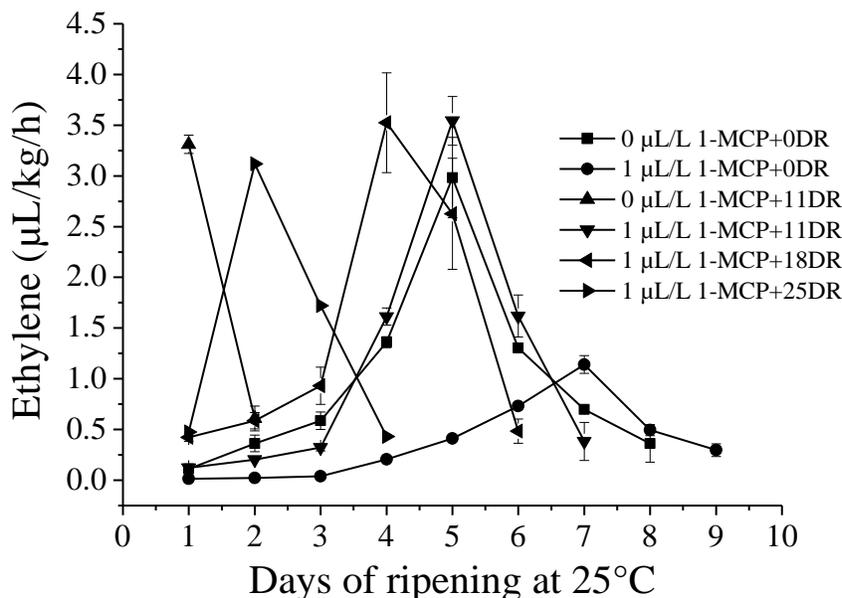


Figure 1. Ethylene production of sapodilla fruit treated with 1-MCP, refrigerated for 0, 11, 18 and 25 days at 16°C and ripened at 25°C. Average values of two replications \pm standard deviation. DR: days of refrigeration.

°Brix. TA was determined by titrating 5 mL of juice with 0.1 N NaOH, to pH 8.1 and expressed as grams of malic acid per 100 g. Reducing sugars were determined following the colorimetric method described by Nelson (1944) and Somogyi (1952) and expressed as grams of glucose per 100 g of pulp.

Pulp color was measured with a Minolta portable colorimeter CR-200 (Minolta Co; Ltd., Osaka, Japan). Two measurements in the equatorial area of the pulp fruit were carried out, using ten fruits per replication. The parameters 'L*', 'a*' and 'b*' were measured and the final results were expressed as hue angle ($h = \arctan[b^*/a^*]$) (McGuire, 1992).

Fruit firmness was determined on whole and unpeeled fruits using an Instron Universal Testing Instrument (Model 4422, Canton, MA, USA) fitted with a flat-plate probe (5 cm diameter) and 50 kg load cell. The force was recorded at 5 mm deformation and was determined at two equidistant points on the equatorial region of each fruit. Ten fruits per treatment were used. The mean values of the firmness were expressed as Newton (N).

Pectin methyl esterase (PME) activity in the fruit was also determined using the method of Ranganna (1979) by the reaction of a sample (4 mL) of enzymatic extract (20 g pulp + 50 mL NaOH 0.2N, pH 7.5) centrifuged (12 500 xg at 4°C for 25 min), with 30 mL of citrus pectin (1%) as substrate and adjustment of pH to 7.5, and the methoxyl groups released by the enzyme per gram of soluble solids per minute (mg methoxyl/g/min) were quantified. Five fruits per treatment were used.

Statistical analysis

For respiration rate and ethylene production two replicates per treatment were used with three fruits per each replica, while for titratable acidity, total soluble solids, reducing sugars and activity of

pectin methyl esterase enzyme (PME) two replicates per treatment were used with five fruits per each replica.

For physiological loss of weight, percentage of ripe sapodilla, color, luminosity of the pulp and firmness two replicates per treatment were used with 10 fruits per each replica. The physiological loss of weight and percentage of ripe fruit were plotted with program Sigma Plot for Windows® V. 2.01, indicating the average and standard deviation. The data obtained for the other variables were statistically analyzed by the analysis of variance (ANOVA) using a completely randomized design and the means were compared with Tukey's multiple-range test at $P < 0.05$ level.

RESULTS AND DISCUSSION

Ethylene production rates

Maximum ethylene production of the fruits treated with 1-MCP, without exposure to refrigeration but with subsequent ripening at 25°C, was significantly lower in comparison with the control fruit, in which the climacteric peak of ethylene appeared on the seventh day with 1.14 $\mu\text{L/kg/h}$ for the treated fruit and on the fifth day with 2.98 $\mu\text{L/kg/h}$ for the control (Figure 1). This would indicate that the treatment with 1-MCP delayed the climacteric maximum by two days in the treated fruit, in comparison with the untreated fruit. The effect of 1-MCP may be a result of the compound blocking the sites of ethylene action at cell level, thereby impeding the perception of the phytohormone and the concomitant gene expression which

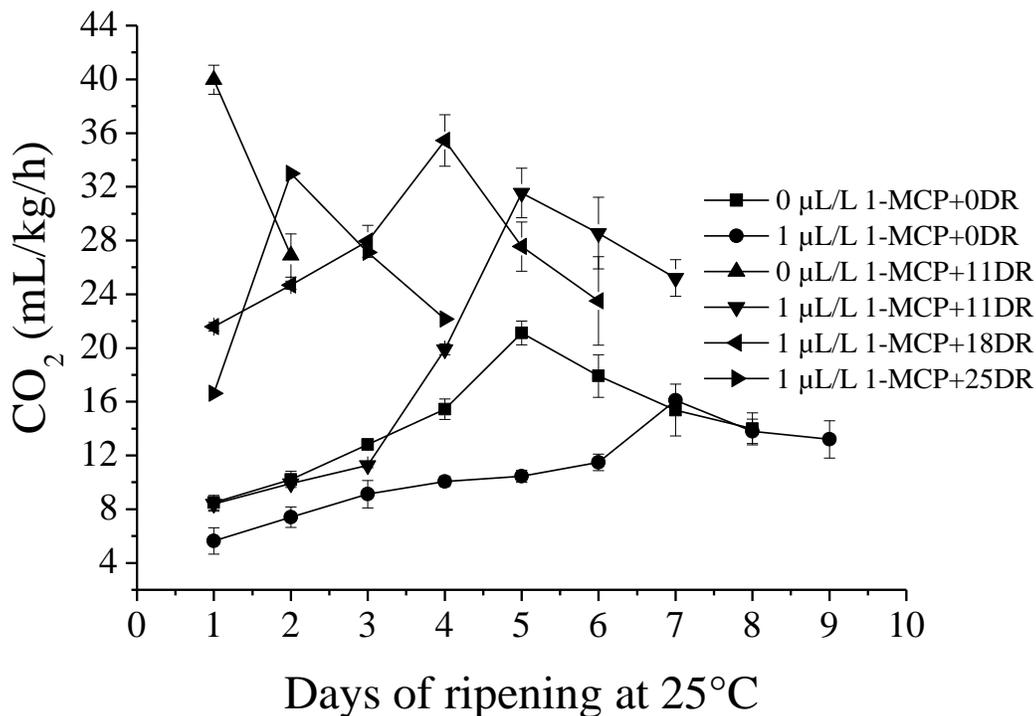


Figure 2. Production of carbon dioxide in sapodilla fruit treated with 1-MCP, refrigerated for 0, 11, 18, and 25 days at 16°C and ripened at 25°C. Average values of two replications \pm standard deviation. DR: days of refrigeration.

participate in its biosynthesis (Binder and Bleecker, 2003). In this sense, other studies have shown that 1-MCP not only delays the appearance of the ethylene peak in fruits such as avocado (Zhang et al., 2013), banana (Zhang et al., 2006), sapodilla (Qiuping et al., 2006) and persimmon (Luo, 2007), but also reduce ethylene production in fruit species such as pears (Villalobos-Acuña et al., 2011), apples (Watkins and Nock, 2012) and mangosteen (Piriyavinit et al., 2011).

After 11 days of storage at 16°C, a similar result was obtained for the ethylene climacteric peak in fruits treated with 1-MCP (3.54 μ L/kg/h) and the control (3.31 μ L/kg/h) when they were compared at 5 and 2 days of exposure to the ripening conditions (25°C), in the same order (Figure 1). In this case, the only observation was that the treatment with 1-MCP significantly delayed the climacteric maximum by 3 days, in comparison with the control.

It is important to note that the untreated fruit reached ripeness for consumption in cold storage (16°C) after 16 days, suggesting advances in the ripening process during refrigeration.

As the period of refrigeration was extended, ethylene production in the fruit treated with 1-MCP increased to 3.52 μ L/kg/h (18 days at 16°C) and 3.12 μ L/kg/h (25 days at 16°C) when they were transferred to the fourth and

second day of ripening, respectively. From this we can assume that the treatment followed by refrigeration prolongs the shelf life of the sapodilla fruit.

These results indicate that the treatment with 1-MCP influences the climacteric maximum of ethylene, thereby prolonging the shelf life of sapodilla fruit in ripening conditions after a period in cold storage.

Respiration rates

According to the results obtained for carbon dioxide production (Figure 2), the maximum respiratory rate recorded for the fruit treated with 1-MCP without cold storage was significantly lower (16.10 mL/kg/h) on the seventh day of ripening at 25°C in comparison with untreated fruit (21.12 mL/kg/h on the fifth day), indicating that the treatment was responsible for delaying maximum respiratory intensity in the fruit by two days as compared to the respective controls.

Moreover, maximum production of carbon dioxide in treated fruit (31.55 mL/kg/h on the fifth day of ripening) after 11 days of refrigeration was lower in comparison with untreated fruit (39.97 mL/kg/h on the first day of ripening) (Figure 2). This would suggest that the compound

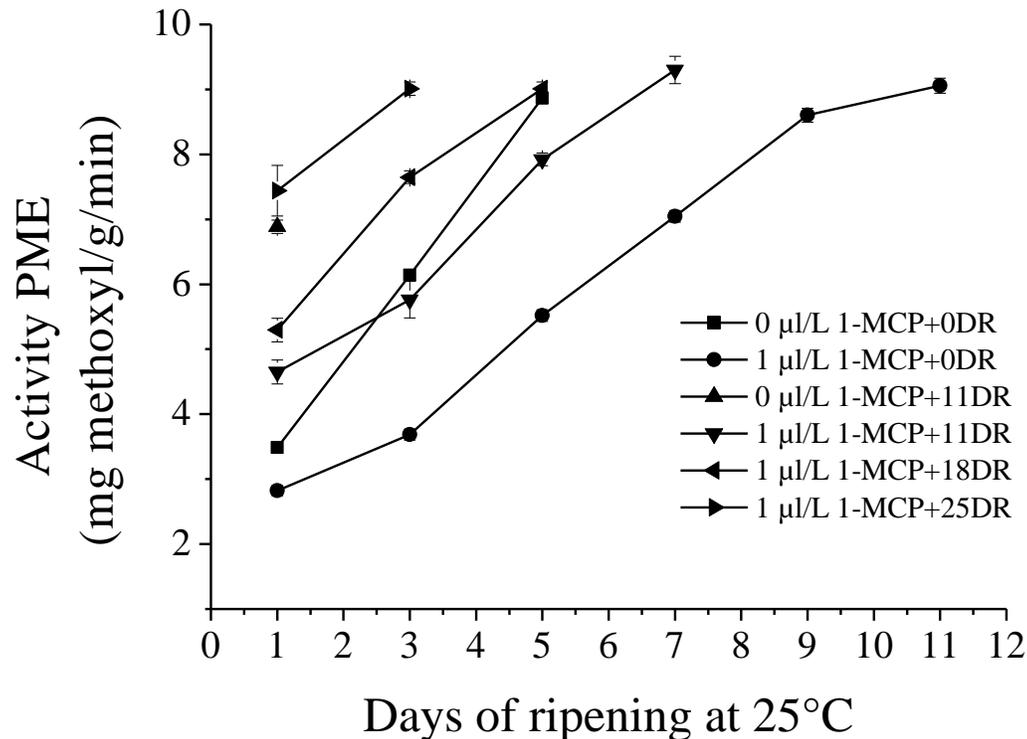


Figure 3. Activity of the pectin methyl esterase enzyme (PME) in sapodilla fruit treated with 1-MCP, refrigerated for 0, 11, 18 and 25 days at 16°C and ripened at 25°C. Average values of two replications \pm standard deviation. DR: days of refrigeration.

caused a reduction in carbon dioxide production and a delay of 4 days in the maximum respiratory rate, in comparison with the control. The evidence appears to indicate that changes in the respiratory intensity of the fruit are dependent on ethylene action (Golding et al., 1998), which is linked to fruit and vegetable deterioration; therefore, a delay or reduction in the respiration rate prolongs the shelf life of fruits (Perera et al., 2003), a situation which is similar to that of this work. The reduction of respiratory intensity in fruit and vegetables due to the action of 1-MCP has been observed in a number of studies for example in banana (Zhang et al., 2006), avocado (Jeong et al., 2002), sapodilla (Qiuping et al., 2006) and persimmon fruit (Luo, 2007). Moreover, the maximum respiration of avocados was delayed for 6 days and reduced in magnitude by approximately 40% due to the treatment with 1-MCP (Blankenship and Dole, 2003).

After the extended refrigeration period, treated fruits showed similar values of respiratory maximums; 35.44 mL of carbon dioxide/kg/h (18 days at 16°C) on day four of the ripening period and 32.8 mL of carbon dioxide/kg/h (25 days at 16°C) on day two, resulting in a longer shelf life for sapodilla fruit.

These results suggest, therefore, that treatment with 1-

MCP had an effect on the respiratory metabolism of sapodilla fruits after cold storage, thereby prolonging their shelf life and facilitating their commercialization.

Enzymatic activity of pectin methyl esterase (PME)

It is true that the fruit softening process is a consequence of the de-esterification of the pectin catalyzed by PME, followed by a depolymerization catalyzed by PG (Abu-Goukh and Bashir, 2003). In this sense, as can be seen in Figure 3, the maximum PME activity in fruits treated with 1-MCP (9 mg methoxyl/g/min on day 11 of ripening), without cold storage and directly exposed to ripening at 25°C, was similar to controls (9.1 mg methoxyl/g/min on day 5 of ripening). However, this maximum PME activity was delayed (6 days) in treated fruit, in comparison with untreated fruit. It is interesting to note that this maximum enzymatic activity coincided with the fruits reaching ripeness for consumption, which would suggest that the treatment was able to delay the ripening process of the sapodilla fruit. Selvaraj and Pal (1984) and Bautista-Reyes et al. (2005) also found increased PME activity in sapodilla during the ripening process, which reached its maximum when the fruit was ripe for consumption. Moreover,

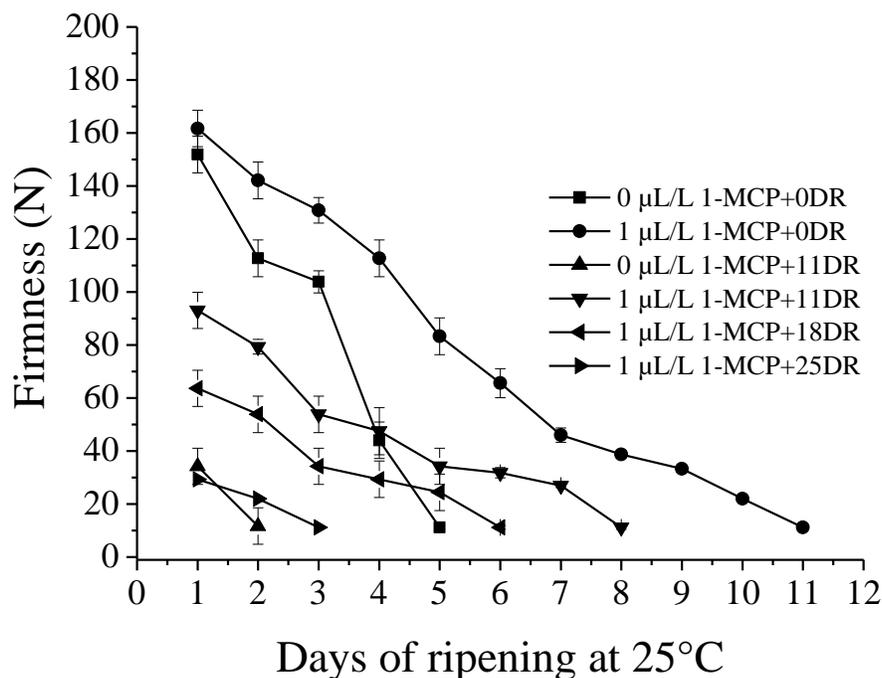


Figure 4. Firmness of sapodilla fruit treated with 1-MCP, refrigerated for 0, 11, 18 and 25 days at 16°C and ripened at 25°C. Average values of two replications \pm standard deviation. DR: days of refrigeration.

after 11 days at 16°C and a subsequent ripening period at 25°C, the treated fruits also required more time to reach maximum PME activity (9.3 mg methoxyl/g/min on the seventh day) as compared to the controls (6.9 mg methoxyl/g/min on the first day) (Figure 3). The evidence suggests that 1-MCP was able to prolong shelf life by delaying ripening. Similarly, Jeong et al. (2002) found that the maximum PME activity was delayed in avocados treated with 1-MCP, in comparison with the control, while, Morais et al. (2008) observed that the treatment with 300 nL/L of 1-MCP in sapodilla resulted in a significant delay of maximum PME activity, when compared the untreated fruit.

Furthermore, after 18 and 25 days in refrigeration, the fruits treated with 1-MCP showed similar values for maximum PME activity on reaching ripeness for consumption (Figure 3).

These results demonstrated that the treatment with 1-MCP exerted considerable influence on the maximum activity of the PME enzyme, thereby prolonging the shelf life of sapodilla with the aim of facilitating the commercialization of this species.

Firmness

The firmness of treated and untreated fruits, without expo-

sure to refrigeration, decreased to values of 11.27 N at 11 and 5 days of ripening, respectively, from which we can assume that loss of firmness was delayed for up to six days in treated fruit in comparison with untreated fruit (Figure 4). It is important to note that the value of firmness (11.27 N) coincided with the fruit reaching ripeness for consumption. A similar response was observed after 11 days in refrigeration. This situation would suggest that the delay in loss of firmness of the treated fruits with 1-MCP may be due to the inhibition of hydrolysis in enzymes such as polygalacturonase (PG), cellulase and pectin methyl esterase (Jeong et al., 2002). Excessive loss of firmness is known to be one of the main limiting factors of the post harvest shelf life of climacteric fruits (Skog et al., 2003). It has been reported that 1-MCP maintains firmness in climacteric fruits (Blankenship and Dole, 2003), a situation which, in the case of the sapodilla, is confirmed by the number of days required to reach a firmness in relation to ripeness for consumption. In other studies, 1-MCP has been shown to delay the loss of firmness in Mexican plum and summer squash (Osuna-García et al., 2011; Massolo et al., 2013).

Similarly, Morais et al. (2008) found that sapodilla fruit treated with 300 nL/L of 1-MCP, presented a slower rate of softening in comparison with untreated fruit.

On the other hand, the firmness of treated fruit decreased

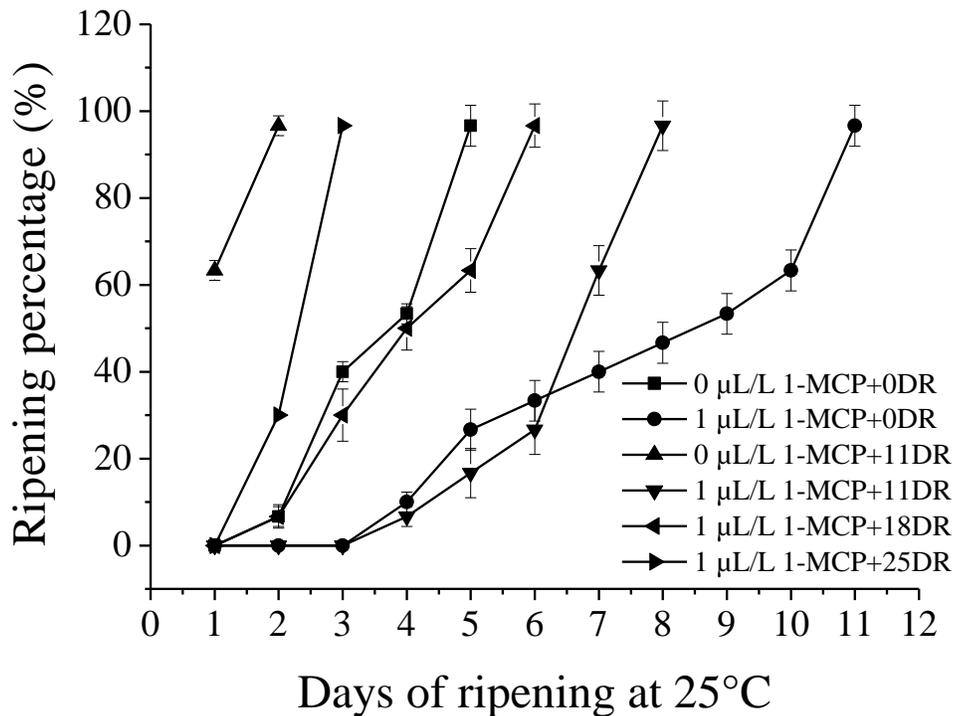


Figure 5. Percentage of ripe sapodilla fruit treated with 1-MCP, refrigerated for 0, 11, 18 and 25 days at 16°C and ripened at 25°C. Average values of two replications \pm standard deviation. DR: days of refrigeration.

to 11.27 N on the sixth and third day of ripening, after 18 and 25 days in refrigeration, respectively. This result indicates that, as the period of refrigeration was extended, the loss of firmness in the fruit was correspondingly faster during the ripening period at 25°C (Figure 4).

In general, the fruit treated with 1-MCP maintained higher values of firmness during the ripening process in comparison with untreated fruit; however, at the end of the ripening period similar values of firmness were obtained with no significant differences. In this sense, one of the advantages of maintaining higher values of firmness is that this reduces the risk of mechanical damage to the fruit (Perez-Vicente et al., 2002), which is one of the most important causes of quality loss in fruit production (Amorim et al., 2008).

Ripening of sapodilla

The fruits treated with 1-MCP, without exposure to refrigeration, required more time to reach ripeness for consumption at 25°C (11 days), in comparison with untreated fruit (5 days), with no changes in the percentage of ripening (91.6-95.8%) (Figure 5). This evidence indicates that 1-MCP significantly delayed ripening up to six days.

After 11 days of refrigeration, the fruit treated with 1-

MCP showed a delay in ripening of 6 days when compared with the untreated fruit, confirming the efficiency of the compound in delaying the ripening process of sapodilla. In relation to this, 1-MCP is a compound that is capable of preventing ethylene action and has also shown to be effective in reducing the post harvest deterioration rate of fruits (Menniti et al., 2004), and thus represents an enormous potential for extending the shelf life of a diversity of fruits. Moreover, it has also proved effective in delaying ripening in pears (Liu et al., 2005), sapodilla (Morais et al., 2006), banana (Pelayo et al., 2003), jujube (Li et al., 2011) and papaya (Moya-León et al., 2004).

After extending the refrigeration period to 18 and 25 days, the treated fruit reached ripeness for consumption on the sixth and third days at 25°C, respectively. Thus, we can affirm that the treatment and refrigeration prolonged the shelf life of this fruit up to 28 days (Figure 5).

Physiological loss of weight

Figure 6 shows that the treatment with 1-MCP in fruit samples that were not refrigerated resulted in a reduction in weight loss on the third (4.55%) and fifth day (7.91%) of ripening at 25°C, in comparison with untreated fruit (5.43% for day 3 and 9.24% for day 5). Similarly, after 11

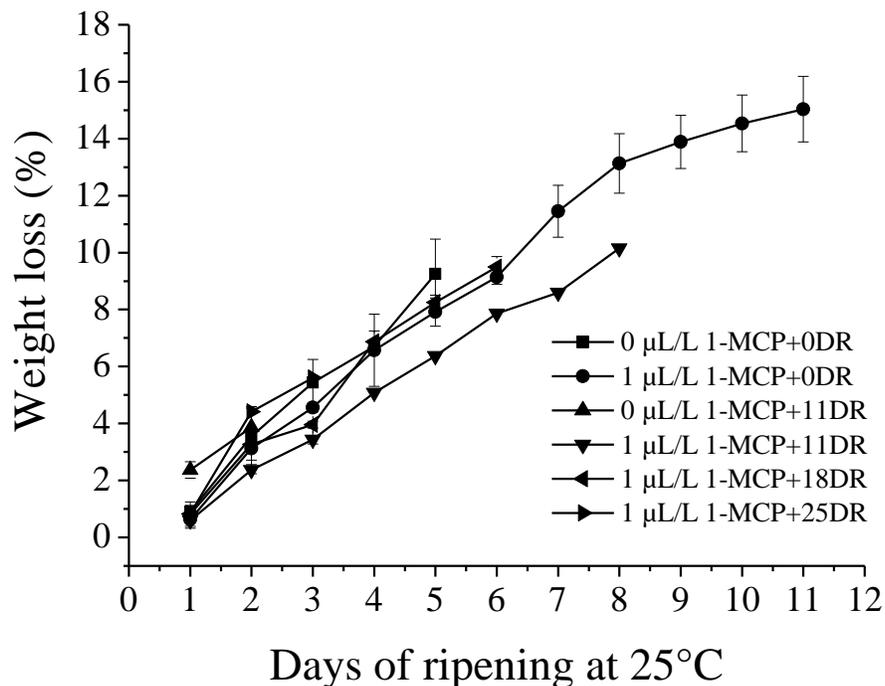


Figure 6. Percentage of weight loss in sapodilla fruit treated with 1-MCP, refrigerated for 0, 11, 18 and 25 days at 16°C and ripened at 25°C. Average values of two replications \pm standard deviation. DR: days of refrigeration.

days of refrigeration, the fruit treated with 1-MCP also showed a reduction in weight loss on day 1 (0.59%) and 2 (2.36%) of ripening at 25°C, as compared to untreated fruit (2.37% for day 1 and 3.87% for day 2). When the treated fruit reached ripeness for consumption, weight loss was greater in comparison with untreated fruit, indicating that the treatment with 1-MCP only reduced weight loss for short periods during the ripening process at 25°C.

In another result, the fruits which were treated and refrigerated at 16°C for 18 days presented lower values of weight loss on day 2 (3.27%) and on day 3 (4.42%) of ripening, as compared to treated fruits which were refrigerated for 25 days (4.42% on day 2 and 5.60% on day 3), indicating that weight loss increased as the refrigeration period was extended for treated fruits with 1-MCP (Figure 6).

It is important to point out that results obtained in other studies suggest that the effect of 1-MCP on weight loss may differ. In some cases, it was observed that 1-MCP had no effect on weight loss, as reported by Franco-Rosa et al. (2013) for orange. In contrast, Osuna-García et al. (2011) reported that 1-MCP reduced weight loss in Mexican plum.

The results of this study indicate that the post harvest application of 1-MCP to sapodilla fruit had an influence on the reduction of weight loss but only for short periods

during the ripening process at 25°C.

Effect of 1-MCP on the main physico-chemical characteristics of sapodilla

When treated and untreated fruits reached ripeness for consumption at 25°C, after refrigeration for 0, 11, 18 and 25 days, no statistically significant differences were observed in titratable acidity (0.11-0.12 g of malic acid/100 g), reducing sugars (9.9-10.0 g of glucose/100 g), pulp color measured as hue angle (79.5-80.3°hue) and lightness (50.0-50.8 L*) (Table 1). In relation to this, a number of studies have reported that 1-MCP does not affect the evolution of acidity in fruits such as apples and oranges (Salvador et al., 2003; Franco-Rosa et al., 2013). It does not affect the change in color of fruits such as apricots, plums and berries (Dong et al., 2002; Gong et al., 2002). Treatment with 1-MCP has also been shown to have no effect on sugar content in summer squash (Massolo et al., 2013), indicating that 1-MCP does not affect sugar metabolism (Salvador et al., 2003).

On the other hand, the total soluble solids content of treated fruit was significantly higher (between 21.0 and 21.5 °Brix) when compared with that of untreated fruit (19.5 °Brix) (Table 1). This increase in soluble solids

brought about by 1-MCP has also been observed in papaya (Hofman et al., 2001). We might infer, therefore, that this effect on treated fruits could be attributed to their low respiration rate; however, we must keep in mind that it also depends on the cultivation and storage conditions (Blankenship and Dole, 2003).

The results obtained in this study indicate that the only significant effect of the post harvest application of 1-MCP to sapodilla was an increase in the total soluble solids content (°Brix) of treated fruit, without affecting the other physico-chemical characteristics of sapodilla pulp. The similar values observed for the characteristics evaluated in ripe fruit, both treated and untreated, indicate that, although 1-MCP delays the physiological activity of sapodilla fruit, the ripening process undergoes virtually no modifications, resulting in a fruit product with normal characteristics of quality.

Conclusions

In general, we can conclude that the treatment with 1-MCP (1 µL/L) and subsequent refrigeration at 16°C resulted in a prolongation of the post harvest shelf life of sapodilla fruit of up to 28 days (25 days at 16°C and 3 additional days in ripening conditions at 25°C). Furthermore, the treatment with 1-MCP significantly reduced the climacteric maximum and respiration rate of mature fruit at 25°C after refrigeration and also delayed the onset of their respective maximums, from 1 to 7 days.

In the fruit treated with 1-MCP, maximum activity of the PME enzyme was reached over a longer period of time, in comparison with untreated fruit, while the treated fruit also maintained greater values of firmness. However, when the fruit reached ripeness for consumption, the values of PME activity and firmness were similar in all the treatments.

The ripening process of both treated and untreated fruits proceeded normally and in a similar fashion, the only details of note is that 1-MCP significantly reduced physiological loss in weight over short periods during ripening and provoked a slightly significant increase in total soluble solids, from which we can affirm that 1-MCP, combined with refrigeration, is an adequate alternative for increasing the shelf life of sapodilla with the aim of facilitating its commercialization.

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Full Length Research Paper

An overview of human-plant interaction in Nikyal rangeland district Kotli Azad Jammu and Kashmir

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The aim of this study was to document the local uses of important plant species of Nikyal valley particularly medicinal, fodder, fuel, timber, fruit, vegetable, tanning, dying and furniture uses. A total of 111 species belonging from 51 families were found to have a variety of uses by the local people for the accomplishment of their basic needs. These species includes 9 tree 20 shrubs and 82 herbs. The major bulk of 52 plant species (35%) were used as fodder/forage. Fifty species (34%) has medicinal uses, 15 species (10%) were used as a fuel wood and 5 (3%) for timber. Eight species (5%) were used as vegetable or edible fruit, three species (2%) each were used for preparation of furniture and tanning or dying, only 2 species (1.5%) were used for resin and three species (1.5%) were used for the preparation of furniture. *Pinus roxburghii*, *Punica granatum*, *Quercus dlatata*, *Olea ferruginea*, etc. have multiple uses such as fodder, timber wood, fuel wood and medicine. These plants are used in individual form or in combination with other species or other edible items. The study revealed that most of the plants are used for medicinal and fodder purposes. The major utility of this ecosystem is for pasture; therefore efforts should be directed to improve the area as a rangeland.

Key words: Nikyal hills, ethnobotany, human plant interaction, conservation.

INTRODUCTION

Ethnobotany focuses on the uses of plants which is established by a particular community over generations. It plays an important role in understanding the dynamic relationships between biological diversity and social and cultural systems (Husain et al., 2008; Mahmood et al., 2011a). It is a multidisciplinary science that studies "the relationship between a given society and its environment and in particular the plant world". People understand and collect the knowledge of valuable plants by the use of anthropological methods (Ajaib et al., 2010). Human interactions with plants vary due to their uses, relative importance and varying social, cultural and ethnic factors (Pahnwar and Abro, 2007). Man have always been dependent on plants for food, shelter, health fragrance, cosmetics, dyeing agent, soap and body care since the pre-historic times. Human being and plant populations have

direct interaction through culture, belief, dependence, economy and commerce (Dashora, 2006).

Nikyal Hills are situated in District Kotli, Azad Jammu and Kashmir at an altitude of 1500-1900 m. They are located 30 km away from Kotli towards North. The investigated area lies within longitude 74° 04' to 10' east and latitude 33° 26' to 29' north. It is surrounded by Kotli on the south, on western side by Tatapani, on Northern side by Mender and on the east by Pir-Panjaj (Amjad et al., 2011). The climate of Nikyal valley is of sub tropical humid type with average annual rainfall of 95.60 mm. The maximum rainfall occurs during July amounting to 251.52 mm, while least rainfall occurs during November amounting to 14.44 mm. The hottest months of the year are June and July, with mean daily maximum temperature of

37.69 and 34.82°C, respectively and minimum temperature of 23.61 and 23.62°C, respectively, while the coldest months of year were December and January with mean maximum temperature of 19.99 and 18.09°C, respectively and minimum temperature of 5.49 and 4.41°C, respectively. The average maximum and minimum relative humidity of the area is 79.64 and 30.82%, respectively (Anonymous, 2006). Plants used as fuel and timber and other uses were extremely studied (Kappelle et al., 2000; Gutkowska et al., 2002; Ibrar et al. 2007; Ishtiaq et al., 2007; Ahmad et al., 2006; Durrani and Manzoor, 2006; Okello and Segawa, 2007; Gilani et al., 2003; Hussain et al., 2006; Zabihullah et al., 2006; Wazir et al., 2004; Khan and Khatoon 2007; Khan and Khatoon, 2008). No such studies are however available on plants of Nikyal valley. The present study reports on the ethnobotanical important resources from the Nikyal Valley, AJK, Pakistan and analyzes the indigenous traditional knowledge on the utilization of the most commonly used plants.

METHODOLOGY

The research was conducted in 12 villages which were selected after general survey and preliminary discussion. For data collection, 60 informers which are native to the area, mainly farmers, shepherds and housewives were interviewed. They were randomly selected during 2012-2013. The informants were aware of the aims and the end use of informations they provided. They also cooperated in collecting and recognizing plants. Moreover, they also indicated where plants were easily available. The plants species collected during sampling were carefully dried and mounted on herbarium sheets. All the available literature was used for identification of species. Nomenclature of Nasir and Ali (1971-1994) and Ali and Qaisar (1995-2006) were followed.

RESULTS

A total of 111 plant species were recorded from Nikyal valley. There were 34% (50 spp.) medicinal plants, 35% (52 spp.) fodder species, 10% (15 spp.) fuel wood species, 3% (5 spp.) timber wood species, 8% (12 spp.) vegetable/edible fruit species, 5% (7 spp.) poisonous plants, 2% (3 spp.) tanning/dying species, 1% (2 spp.) resin yielding species. Only 2% (3 spp.) were used for furniture making (Table 1 and Figure 1). The data shows that the area is suitable for rangeland and medicinal plants.

DISCUSSION

Interaction between plant and humans is very strong and can never be separated as the dependence is obligate. The plant resources lead to the economical wealth of an area. The utility and use of plants create the importance of plant in that area. In the same context when the plant of Nikyal valley were analyzed, it was observed that all 111 species had different local uses as such medicinal, fuel, fodder and construction purposes. The majority of plant species, that is, 54 (40%) was used as fodder. They had different palatability value. This indicates that area is

well suited for rangeland. Poor vegetation and moist and cold weather conditions cannot support agriculture. Most of the species reported in the present study have also been reported as fodder species by some other worker (Hussain et al., 2004, 2006; Badshah et al., 2006; Kappelle et al., 2000; Tordio et al., 2006; Arenas and Scarpa, 2007; Maoe et al., 2009; Ajaib et al., 2010; Bano et al., 2013).

The next major utility of 63 (47%) was for medicinal purpose. Exploitation of medicinal plants by local folk, collectors and herbal drug dealers was increasing with increasing demand of pharmaceutical industry and non awareness of local inhabitants. This caused drastic decrease in the occurrences and products of medicinal plants. Grazing, browsing, deforestation and soil erosion were mainly responsible for reduction in the medicinal flora. It is therefore essential to have conservation strategies for these medicinal plants. Therefore, the preferred medicinal plants grow at high elevation where man and grazing animal could not reach easily. The increasing population has pressurized the medicinal plant which has dramatically decreased the species and population of medicinal plants (Ajaib et al., 2010). The nomads collect the medicinal plants for their earning. They uproot and collect each part of the medicinal plants in non scientific way. Prior to this study, no reference exist on the medicinal plant of this area. Most species in the present study have also been reported as medicinal by some other worker like Gilani et al. (2003), Wazir et al. (2004), Jabar et al. (2006), Ishtiaq et al. (2007), Hussain et al. (2008), Sardar and Khan (2009), Tareen et al. (2010) and Bano et al. (2013).

Deforestation, overgrazing and soil erosion were the main factor responsible for the reduction of medicinal plant in this area. The local live stocks grazed most of the medicinal plant. It is therefore essential to have conservation strategies for these medicinal plants. The collection of plant must be correlated with the phenological cycle. The plants are abrupt to graze and collected for medicinal or fuel wood purpose. Similarly, the plants grazed or collected for root, rhizome, bulb and flower become more threatened. The shoots fail to develop seed and flowers while the rhizomatous plants are destructively collected. This will reduce the chance of their regeneration.

In the investigated area, most people are poor and lack the basic facilities. They depend upon the forest for fuel wood. There are 15 (10%) species used for fuel wood. These include the *Quercus dilatata*, *Punica granatum*, *Pinus roxburghii*, *Plectranthus rugosus* and *Zanthoxylum alatum*. The use of plants as fuel wood from adjoining areas has been reported by other workers (Sardar and Khan, 2009; Ajaib et al., 2010; Qasim et al., 2010). The furniture wood consisting of *P. roxburghii* is also valuable source of earning to meet their demands. Some of these plants with similar uses have been reported by Qureshi et al. (2007), Hussain et al. (2004) and Shah and Hussain (2008).

Table 1. Contd.

<i>Bupleurum falacatum</i> L.	-	-	-	-	-	-	-	-	-
<i>Chrysopogon aucheri</i> (Boiss) Stapf	-	+	-	-	-	-	-	-	-
<i>Commelina benghaliensis</i> Linn.,sp. Pl.	+	+	-	-	+	+	-	-	-
<i>Conyza ambigua</i> Hook. Arn	+	-	-	-	-	-	-	-	-
<i>Conyza canadensis</i> L.	+	-	-	-	-	-	-	-	-
<i>Cymbopogon jwarancusa</i> (Jones) Schult.	+	+	-	-	-	-	-	-	-
<i>Cynodon dactylon</i> (L.) Pers	-	+	-	-	-	-	-	-	-
<i>Cynoglossum lanceolatum</i> Forssk	-	+	-	-	-	-	-	-	-
<i>Poa inferna</i> H.Bk.	-	+	-	-	-	-	-	-	-
<i>Cyperus globosus</i> Forssk.	+	+	-	-	-	-	-	-	-
<i>Cyperus niveus</i> Retz	+	+	-	-	-	-	-	-	-
<i>Cyperus rotundus</i> L.	+	+	-	-	-	-	-	-	-
<i>Cyprus difformis</i> L.	+	+	-	-	-	-	-	-	-
<i>Dichanthium annulatum</i> (Forssk).Stapf	-	+	-	-	-	-	-	-	-
<i>Dicliptera roxburghiana</i> Nees	-	+	-	-	-	-	-	-	-
<i>Dryopteris stewartii</i> Fress	-	+	-	-	-	-	-	-	-
<i>Eragrostis japonica</i> Thunb. Trin.	-	+	-	-	-	-	-	-	-
<i>Eriophorum comosum</i> Wall	-	-	-	-	-	-	-	-	-
<i>Euphorbia helioscopia</i> L.	+	-	-	-	-	+	-	-	-
<i>Euphorbia prostrata</i> Ait	-	-	-	-	-	+	-	-	-
<i>Euphorbia wallichii</i> HK..f	-	-	-	-	-	+	-	-	-
<i>Fragaria nubicola</i> Landle ex Lacaita	+	+	-	-	+	-	-	-	-
<i>Galium elegan</i> Wall.	-	+	-	-	-	-	-	-	-
<i>Geranium rotundifolium</i> L.	+	-	-	-	-	-	-	-	-
<i>Gerbera gossypina</i> Royle	-	+	-	-	-	-	-	-	-
<i>Hedra nepalensis</i> K. Koch	-	+	-	-	-	-	-	-	-
<i>Heteropogon contortus</i> (L.)	-	+	-	-	-	-	-	-	-
<i>Hypericum perforatum</i> L.	+	-	-	-	-	-	-	-	-
<i>Ipomea purpurea</i>	+	-	-	-	-	-	-	-	-
<i>Impatiens edgeworthii</i> H.K.f	-	+	-	-	-	-	-	-	-
<i>Imperata cylindrica</i> (L.)	-	+	-	-	-	-	-	-	-
<i>Ipomea cordata</i>	+	-	-	-	-	-	-	-	-
<i>Juncus serotinus</i> Clarke	-	-	-	-	-	-	-	-	-
<i>Justica paploroides</i>	+	+	-	-	-	-	-	-	-
<i>Lespedeza sericea</i> (Thunb) Miq	-	-	-	-	-	-	-	-	-
<i>Malva parviflora</i> L.	-	+	-	-	+	-	-	-	-
<i>Malvestrum coromendelianum</i> L.	-	+	-	-	-	-	-	-	-
<i>Medicago denticulata</i> Willd	+	-	-	-	-	-	-	-	-
<i>Melilotus indica</i> (L) All	+	-	-	-	-	-	-	-	-
<i>Micromeria biflora</i> (Ham) Bth	+	-	-	-	-	-	-	-	-
<i>Myrsine africana</i> L.	-	+	+	+	-	-	-	-	-
<i>Oenothera rosea</i> (L). Her	+	-	-	-	-	-	-	-	-
<i>Origanum vulgare</i> L.	+	-	-	-	-	-	-	-	-
<i>Oxalis corniculata</i> L.	-	-	-	-	+	+	-	-	-
<i>Phalaris arundinacea</i>	-	+	-	-	-	-	-	-	-
<i>Poa annua</i> L.	-	+	-	-	-	-	-	-	-
<i>Polygonum aviculare</i>	+	+	-	-	-	-	+	-	-
<i>Prunella vulgaris</i> L.	+	+	-	-	-	-	-	-	-
<i>Pteris cretica</i> L.	-	+	-	-	-	-	-	-	-

Table 1. Contd.

<i>Ranunculus muricatus</i> L.	+	-	-	-	-	-	-	+	-
<i>Rubia tinctorum</i> L.	+	+	-	-	-	-	+	-	-
<i>Rumex hastatus</i> D.Don	+	-	-	-	-	-	-	-	-
<i>Rumex nepalensis</i> D.Don	+	+	-	-	-	-	+	-	-
<i>Saccharum spontaneum</i> L.	-	-	-	-	-	-	-	-	-
<i>Scutellaria linearis</i> Benth	-	-	-	-	-	-	-	-	-
<i>Setaria viridis</i>	-	+	-	-	-	-	-	-	-
<i>Solanum nigrum</i> L.	+	-	-	-	+	-	-	-	-
<i>Solanum surattense</i> Burm. f.	+	-	-	-	-	-	-	-	-
<i>Sonchus arvensis</i> L.	+	-	-	-	-	-	-	-	-
<i>Sonchus asper</i> Hill	+	-	-	-	-	-	-	-	-
<i>Sorghum halepense</i> (L.)Pers	-	+	-	-	-	-	-	-	-
<i>Taraxacum officinale</i> Weber	+	-	-	-	+	-	-	-	-
<i>Themeda anathera</i> (Hack)	-	+	-	-	-	-	-	-	-
<i>Trifolium repens</i> L.	+	+	-	-	-	-	-	-	-
<i>Tussilago farfara</i> L.	+	-	-	-	-	-	-	-	-
<i>Valeriana jatamansi</i> Jones	+	-	-	-	-	-	-	-	-
<i>Viola odorata</i> L.	+	-	-	-	-	-	-	-	-

M = Medicinal, F = fodder, TW = timber wood, FW = fuel wood, V = vegetable, E = edible fruits, TD = tanning/dying, P = poisonous, RT = roof thatching, FT = furniture.

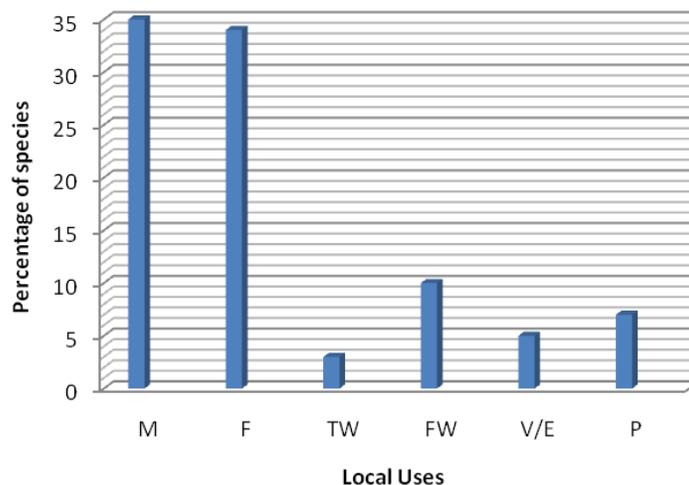


Figure 1. Economic use classification of flora of Nikyal valley.

Timber wood harvest from forest resources has become one of the major ecological problems. In recent times, there is pressure on species for burning and construction material. This has led to creation of barren areas. Plants such as *Contoneaster acuminatus*, *Ficus palmata* and *P. roxburghii* and *Q. dlatata* are highly valuable as timber wood with high selling and buying prices.

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Full Length Research Paper

Species composition, diversity and distribution in a disturbed Takamanda Rainforest, South West, Cameroon

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This study assessed the diversity and distribution of trees and shrubs in a 16 ha disturbed plot in the Takamanda Rainforest. Linear transects (8) were laid in the field which radiated from the centre of the plot. The girth of the trees and shrubs were measured and species identified. A total of 99 species (72 trees and 27 shrubs) belonging to 87 genera and 34 families were recorded. Caesalpiniaceae was the most represented tree family (9 species) while Rubiaceae was the most represented among shrubs (9 species). *Baphia nitida* recorded the highest tree density (143.75 individual's ha⁻¹) and lowest tree density (1.56 individual's ha⁻¹) was recorded for *Khaya anthotera*. The highest shrub density (192.19ha⁻¹) was recorded for *Angylocalyx pynatii* while the lowest was (1.56 ha⁻¹) was recorded for *Voacanga africana*. The girth class distribution showed a reverse J-shape distribution, with the highest densities in the lower girth (15-30 and 30-45 cm) classes. This decreases in density with the larger girth (> 75) class. The Importance Value Index (IVI) of trees was highest for *Baphia nitida* (20.06) while the lowest was recorded for *Millettia sanagana* (0.51). For shrubs, *Chytranthus macrobotrys* had highest IVI of 45.05 while the lowest was observed in *V. Africana* (1.24). Diversity index of trees and shrubs were 3.87 and 2.88, respectively. A dominance index of trees was 0.03 and that of shrubs was 0.08. The species evenness for trees (0.90) and shrubs (0.87) showed a slight variation in distribution. Abundance/frequency ratio (A/F) for tree and shrub was >0.05 and showed a clumped pattern of distribution. Sustainable management of the forest would continue to provide goods and services for communities around the rainforest.

Key words: Diversity index, distribution, abundance/frequency ratio, contagious, importance value index, evenness index.

INTRODUCTION

Tropical rainforest are looked upon as one of the most species-diverse terrestrial ecosystems. They are distinguished from all other terrestrial ecosystems by a very

high diversity at many levels (species, life forms, etc). Their immense biodiversity generates a variety of natural resources which helps to sustain livelihoods of both local

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and urban communities (Kumar et al., 2006). Some of these goods enjoyed by forest dwellers include: medicinal plants, fodder, food, fruits, bush meat, construction materials, etc (Ndah et al., 2013a). Forest communities also enjoyed services provided by the forest, some of which include: the regulation of temperature, purification of air, detoxification of soil, thus producing a healthy environment for livelihoods support (Tripathi et al., 2010; Hadis et al., 2009)

Trees, apart from forming the major structural and functional basis of tropical rainforest, are vital as carbon sinks, water sheds, provide shades and homes to many life forms and above all, act as a primary harvester of energy into the ecosystem (Singh, 2002). Trees diversity is vital to tropical forest biodiversity, because tree provide homes and resources to a wide variety of plant and animal species (Huang et al., 2003). Tree species diversity and spatial distribution in tropical forest are greatly influence by biogeography, niche requirement and disturbance (Huang et al., 2003).

Many tropical forests are undergoing severe anthropogenic modifications such as cutting down of forest for plantation establishment, poor farming techniques, poor hunting and trapping practices (Ndah et al., 2012, 2013a, b). Over exploitation of non-timber forest products, etc and sustainable management techniques are required to maintain the biodiversity and productivity of the ecosystems (Reddy and Ugle, 2008).

The Takamanda Rainforest is an area noted for its richness and diversity in plant and animal species which are widely distributed within the different ecological types (Ndah et al., 2012; Sunderland-Groves, 2003). This area harbours some African threatened species that are of paramount conservation interest. Some of these tree species included *Terminalia ivorensis*, *Pterocarpus soyauxii*, *Milicia excelsa*, *Balonella toxisperma*, *Staudtia stapitata*, *Afzelia bipindensis* and *Diospyros crassiflora* (Sunderland et al., 2003). Besides the plant species, the forest equally harbours animals of conservation interest. Amongst these are the Nigeria-Cameroon chimpanzee (*Pan troglodytes vellerosus*), drill (*Mandrillus leucophaeus*) and Preuss's guenon (*Cercopithecus preussi*) (Grove and Maisel, 1999). The Cross River gorilla (*Gorilla gorilla diehli*) apart from being endangered is endemic to the area (Grove and Maisel, 1999; Ndah et al., 2012).

This rainforest have been subjected to anthropogenic activities, mainly indiscriminate exploitation of timber (*Pterocarpus soyauxii*, *Terminalia ivorensis*, *Eribroma oblongata*) and gathering of non-timber forest products (*Irvingia* spp., *Garcinia manii*, *Carpolobia* spp.) as well as slash and burn system of agriculture (Ndah et al., 2012, 2013a). Nevertheless, the Takamanda rainforest is part

of the Guineo-Congolean forest which harbours some endemic life forms of the world (Lawson, 1996). The control of man's excesses in this ecosystem may support biodiversity conservation.

The aim of this study was to evaluate tree and shrub composition, diversity and distribution pattern in a disturbed Takamanda rainforest. This study gives baseline information on the effects of anthropogenic disturbance on forest species distribution and diversity. This will enable the different stakeholders to take appropriate decisions and measures in sustainable forest management.

MATERIALS AND METHODS

Location of study area

The Takamanda Rainforest which covers a surface area of 67,599 ha is located in the South West Region and this part of Cameroon has been described in details in Ndah et al. (2012). Rainfall in this forest has a range of 2500 to 4000 mm per annum (Egbe Enow Andrew, unpublished data) and gives rise to a diverse floristic composition. The low land forest is dominated by *Afrostryas kamerunensis*, *Klainedoxa gabunensis* and *Irvingia gabonensis*, the montane forest dominated by *Xylopia staudtii*, *Macaranga occidentalis* and *Bridelia grandis*; the grassland dominated by *Hyparrhenia diplondra*, *Setaria ancep* and *Loudetia camerunensis* (Sunderland et al., 2003).

The terrain is undulating in the lowland areas, but rises sharply to an altitude of 1,500 m in the northern part of the rainforest, where slopes are extremely steep. In general, the region has two distinct seasons with most rainfall occurring from April to November, with peak period in July-August and September. The mean annual temperature is about 27°C. Normally, it is cooler in the rainy season than in the dry season (Comiskey et al., 2003). The estimated human population of the area is between 6 and 12 individuals per km² (Ndah et al., 2013a).

Vegetation assessment

Eight linear transects 500 m long running north, northeast, east, southeast, south, southwest, west and northwest were established in the disturbed forest radiating from the centre of the site. Radiating transects traversed swamps, plains, ridges, slopes, gully and valleys. A quadrat size of 20 x 20 m was used for trees, 5 x 5 m for shrubs. Girth was measured at breast height (DBH 1.3 m) using a girth tape. In the case of buttressed trees, the measurements were made above the buttress. All trees in the plot were enumerated and identified with the help of Flora of West Africa (Hutchinson and Dalziel, 1954) and voucher specimens of unidentified species were taken to the Limbe Botanical Garden (SCA) for identification. To determine girth class sizes, only tree species with girth ≥ 15 cm was used for the analysis. For the shrubs girth size of ≥ 1 and ≤ 10 cm were used for the analysis.

Data analysis

Frequency, density, basal area, abundance and importance value index (IVI) of plant species were calculated following Misra (1974) and Mueller-Dombois and Ellenberg (1974). The basal area of each

Table 1. Density, basal area, importance value index (IVI) and abundance frequency ratio (A/F) of trees in the study area.

Specie	Family	Density ha⁻¹	Basal area (cm² ha⁻¹)	IVI	A/F ratio
<i>Azelia africana</i>	Mimosaceae	1.56	191.65	0.85	0.49
<i>Albizia adianthifolia</i>	Mimosaceae	7.81	1701.93	4.88	0.81
<i>Albizia zygia</i>	Leguminosae	54.69	2653.36	15.27	1.21
<i>Allanblackia floribunda</i>	Guttiferae	7.81	302.34	2.38	0.81
<i>Alstonia boonei</i>	Apocynaceae	1.56	10780.33	19.69	0.49
<i>Amphimas pterocarpoides</i>	Papilionaceae	18.75	277.90	4.86	0.83
<i>Annickia chlorantha</i>	Annonaceae	15.63	20.43	3.39	0.97
<i>Anthonotha macrophylla</i>	Moraceae	29.69	31.56	6.60	0.92
<i>Antiaris africana</i>	Moraceae	31.25	147.74	7.31	0.88
<i>Baphia nitida</i>	Papilionaceae	143.75	48.99	20.06	3.19
<i>Barteria fistulosa</i>	Passifloraceae	23.44	106.47	5.73	0.81
<i>Bertiera fistulosa</i>	Rubiaceae	3.13	155.24	1.29	0.49
<i>Bridelia micrantha</i>	Euphorbiaceae	10.94	676.34	3.72	0.85
<i>Calpocalyx dinklagei</i>	Caesalpiniaceae	42.19	231.27	7.60	1.64
<i>Canarium schweinfurthii</i>	Burseraceae	1.56	20.87	0.54	0.49
<i>Canthium arnoldianum</i>	Rubiaceae	3.13	225.62	1.41	0.49
<i>Carapa procera</i>	Meliaceae	4.69	41.74	1.59	0.49
<i>Ceiba pentandra</i>	Bombacaceae	4.69	2944.98	6.76	0.49
<i>chumanyophytum sp</i>	Rubiaceae	4.69	18.42	0.87	1.46
<i>Coelocaryon preussii</i>	Myristicaceae	3.13	185.94	1.00	0.97
<i>Cola millenii</i>	Sterculiaceae	31.25	49.99	6.46	1.08
<i>Dacryodes edulis</i>	Burseraceae	6.25	132.82	1.92	0.65
<i>Dialium lopens</i>	Caesalpiniaceae	1.56	155.24	0.78	0.49
<i>Diospyros herienasis</i>	Ebenaceae	1.56	66.71	0.62	0.49
<i>Distemonanthus benthamianus</i>	Caesalpiniaceae	10.94	1360.41	5.28	0.68
<i>Drypetes preussii</i>	Euphorbiaceae	29.69	22.71	6.92	0.84
<i>Eriroma oblongata</i>	Sterculiaceae	1.56	3880.92	7.41	0.49
<i>Eriocoelum macrocarpum</i>	Sapindaceae	1.56	155.24	0.78	0.49
<i>Ficus exaspirata</i>	Moraceae	25.00	401.56	6.42	0.86
<i>Ficus mucoso</i>	Moraceae	4.69	1466.98	3.79	0.73
<i>Funtumia africana</i>	Apocynaceae	26.56	217.98	6.94	0.75
<i>Garcinia staudtii</i>	Guttiferae	3.13	4.91	0.68	0.97
<i>Greenweodendron sp</i>	Annonaceae	3.13	185.94	1.34	0.49
<i>Grewia coriacea</i>	Tiliaceae	1.56	1.92	0.51	0.49
<i>Hannoa klaineana</i>	Simaroubaceae	6.25	240.41	2.11	0.65
<i>Hyloidendron gabunense</i>	Caesalpiniaceae	15.63	30.21	3.41	0.97
<i>Hypodaphnis zenkeri</i>	Caesalpiniaceae	3.13	30.66	1.07	0.49
<i>Irvingia gabonensis</i>	Irvingiaceae	6.25	789.77	3.43	0.49
<i>Khaya anthotera</i>	Caesalpiniaceae	1.56	155.24	0.78	0.49
<i>Lannea welwichii</i>	Anacardiaceae	7.81	1500.39	4.52	0.81
<i>Macaranga monandra</i>	Euphorbiaceae	9.38	196.80	3.04	0.58
<i>Maesopsis eminii</i>	Rhamnaceae	4.69	913.59	3.14	0.49
<i>Margaritaria discoidea</i>	Euphorbiaceae	1.56	2767.43	5.43	0.49
<i>Milicia excelsa</i>	Moraceae	3.13	2944.98	6.25	0.49
<i>Millettia mannii</i>	Papilionaceae	6.25	294.68	1.87	0.97
<i>Millettia sanagana</i>	Papilionaceae	1.56	1.92	0.51	0.49

Table 1. Contd.

<i>Musanga cecropioides</i>	Cecropiaceae	15.63	661.61	4.87	0.81
<i>Myrianthus arboreus</i>	Cecropiaceae	32.81	155.48	6.47	1.28
<i>Newbouldia laevis</i>	Bignoniaceae	3.13	76.07	0.81	0.97
<i>Oncuba gloca</i>	Euphorbiaceae	1.56	191.65	0.85	0.49
<i>Pentaclethra macrophylla</i>	Mimosaceae	6.25	124.19	1.90	0.65
<i>Plagiostyles africana</i>	Euphorbiaceae	21.88	545.52	4.99	1.36
<i>Pseudospondias microcarpa</i>	Anacardiaceae	4.69	1295.56	3.14	1.46
<i>Pterocarpus soyauxii</i>	Leguminosae	32.81	342.91	7.14	1.13
<i>Pycnanthus angolensis</i>	Myristicaceae	12.50	603.82	4.10	0.78
<i>Ricinodendron heudelottii</i>	Euphorbiaceae	18.75	6081.92	16.21	0.58
<i>Santiria trimera</i>	Burseraceae	6.25	264.60	1.81	0.97
<i>Scottellia sp.</i>	Flacourtiaceae	1.56	460.44	1.32	0.49
<i>Sorindeia grandifolia</i>	Anacardiaceae	1.56	18.42	0.54	0.49
<i>Staudtia kamerunensis</i>	Myristicaceae	3.13	141.74	0.92	0.97
<i>Sterculia tragacantha</i>	Sterculiaceae	75.00	321.66	13.61	1.56
<i>Tabernaemontana crassa</i>	Apocynaceae	1.56	68.99	0.63	0.49
<i>Thorianthus sp</i>	Meliaceae	1.56	78.50	0.65	0.49
<i>Treculia obovoidea</i>	Moraceae	4.69	21.29	1.55	0.49
<i>Trichilia rubescens</i>	Miliaceae	29.69	264.95	7.01	0.92
<i>Trichilia welwitschii</i>	Miliaceae	3.13	931.81	2.67	0.49
<i>Trilepisium madagascariense</i>	Moraceae	10.94	328.53	3.44	0.68
<i>Uapaca guineensis</i>	Euphorbiaceae	1.56	766.60	1.87	0.49
<i>Vitex grandifolia</i>	Vitaceae	1.56	3066.41	5.96	0.49
<i>Xylopi aethiopica</i>	Annonaceae	17.19	321.18	4.43	0.89
<i>Xylopi quintasii</i>	Annonaceae	3.13	8.25	0.69	0.97
<i>Zanthoxylum xylate</i>	Rutaceae	3.13	328.89	1.26	0.97
		945.3125	56203.49	300	

individual tree species was calculated as $Ba = \pi (1/2 Dbh)^2$

The importance value index (IVI) for trees and shrubs were calculated by summing the relative frequency, relative density and relative dominance for trees and shrubs. Importance value index was calculated from the values of relative frequency and relative density.

The species diversity index was calculated following Shannon-Wiener index (1963), where: $H' = -\sum (ni/N) \ln ni/N$ and H' = Shannon-Wiener index of general diversity, ni = importance value index of i^{th} species, N = sum of importance value index of all the species.

The species dominance index was calculated by the formula given by Simpson (1949) $Cd = \sum (ni/N)^2$, ni = importance value index of i^{th} species, N = sum of importance value index of all the species.

The spatial distribution of trees and shrubs was determined following Whitford (1949): $WI = \text{abundance/frequency (A/F Ratio)}$. A value <0.025 would imply a regular distribution, values between $0.025-0.05$ means a random distribution and a value >0.05 would mean a contagious distribution.

RESULTS

Tree species composition

A total of 99 tree species belonging to 87 genera under 34 families were recorded at the study area (Table 1). Seventy-two (72) tree (height above 10 meters) species were recorded in the study site (Table 1). Most of the species (9) belonged to the family Caesalpiniaceae followed by Moraceae (7 species), Meliaceae and Papilionaceae (6 species each), Annonaceae and Sterculiaceae (4 species each), Anacardiaceae, Apocynaceae, Burseraceae, Euphorbiaceae, Leguminosae, Mimosaceae and Myristicaceae (3 species each), Cecropiaceae, Guttiferae and Pandaceae (2 species each) Bignoniaceae, Bombacaceae, Ebenaceae, Flacourtiaceae, Irvingiaceae, Passifloraceae, Rhamnaceae, Rutaceae, Sapindaceae and Simaroubaceae (1 species each) (Table 1).

Table 2. Density, basal area, importance value index (IVI) and abundance frequency ratio (A/F) of shrubs in the study area.

Specie	Family	Density (ha ⁻¹)	Basal area (cm ² ha ⁻¹)	IVI	A/F ratio
<i>Angylocalyx oligophyllus</i>	Papilionaceae	14.06	5.25	4.10	1.19
<i>Angylocalyx pynatii</i>	Papilionaceae	192.19	5.43	37.24	2.16
<i>Carpolobia alba</i>	Polygalaceae	6.25	6.04	3.19	0.53
<i>Carpolobia lutea</i>	Polygalaceae	31.25	7.03	13.50	0.48
<i>Chytranthus macrobotrys</i>	Sapindaceae	4.69	307.49	45.05	0.40
<i>Chytranthus tabotii</i>	Sapindaceae	10.94	45.75	11.65	0.37
<i>Coffea sp.</i>	Rubiaceae	3.13	54.86	9.60	0.26
<i>Crateristernum aristatum</i>	Rubiaceae	4.69	17.63	5.38	0.26
<i>Dracaena camerooniana</i>	Dracaenaceae	9.38	1.55	2.20	1.58
<i>Glyphaea brevis</i>	Tiliaceae	7.81	24.02	5.13	1.32
<i>Heinisa crinita (Afzel</i>	Rubiaceae	4.69	27.67	6.78	0.26
<i>Lasianthera africana</i>	Icacinaceae	40.63	5.07	12.90	0.76
<i>Maesobotrya staudtii</i>	Euphorbiaceae	1.56	11.98	2.64	0.26
<i>Mallotus oppositifolius</i>	Euphorbiaceae	125.00	8.88	27.47	1.62
<i>Massularia acuminata</i>	Rubiaceae	6.25	15.29	5.25	0.35
<i>Microdesmis puberula</i>	Papilionaceae	45.31	5.49	11.26	1.27
<i>Microdesmis zenkeri</i>	Papilionaceae	43.75	4.86	14.05	0.74
<i>Pavetta staudtii Hutch</i>	Rubiaceae	9.38	1.92	5.33	0.32
<i>Penianthus camerounensis</i>	Menispermaceae	1.56	2.11	1.27	0.26
<i>Psychotria biferia</i>	Rubiaceae	1.56	8.45	2.15	0.26
<i>Rauvolfia vomitoria</i>	Apocynaceae	21.88	67.44	19.17	0.41
<i>Rinorea digitata</i>	Violaceae	148.44	4.19	31.39	1.67
<i>Rinorea oblongifolia</i>	Violaceae	14.06	17.25	6.54	0.79
<i>Rothmannia hispida</i>	Rubiaceae	4.69	21.72	5.95	0.26
<i>Rothmannia isuda</i>	Rubiaceae	14.06	12.57	5.12	1.19
<i>Rothmannia sp</i>	Rubiaceae	1.56	24.84	4.44	0.26
<i>Voacanga africana</i>	Apocynaceae	1.56	1.92	1.24	0.26
		770.31	716.72	300	

Total stem density in the study area was 1716 individuals' ha⁻¹ which comprised 945.3 and 770.31 for trees and shrubs, respectively (Table 1). The tree species with the highest stem densities was *Baphia nitida* with 143.75 individuals' ha⁻¹ while the lowest was recorded for *Afzelia Africana*, *Alstonia boonei*, *Canarium schweinfurthii*, *Dialium lopens*, *Diospyros herienensis*, *Eriobroma oblongata*, *Eriocoelum macrocarpum*, *Grewia coriacea*, *Khaya anothera*, *Margaritaria discoidea*, *Millettia sanagana*, *Oncuba gloca*, *Scottellia sp.*, *Sorindeia grandifolia*, *Tabernaemontana crassa*, *Thorianthus sp.*, *Uapaca guineensis* and *Vitex grandifolia* with 1.56 individual ha⁻¹ (Table 1).

The basal area ranged from 1.92-10780.33 cm² ha⁻¹ for different species in the study area (Table 1). Total basal area of tree species recorded was 56203.49 cm² ha⁻¹. The highest basal area of 10780.33cm² ha⁻¹ was

observed with *A. boonei*. This was closely followed by *Ricinodendron heudelottii* with basal area of 6081.92 cm² ha⁻¹ (Table 1).

The lowest basal area of 1.92cm² ha⁻¹ was noted with *Millettia sanagana* and *Grewia coriacea*.

The Importance Value Index (IVI) of tree species ranged from 0.51-20.06 (Table 1). *Baphia nitida* had the highest IVI of 20.06 which was closely followed by *A. boonei* with IVI 19.69. This signified that *B. nitida* and *A. boonei* were the most dominant species of the area while *M. sanagana* and *Sorindeia grandifolia* were the least dominant species in the study area (Table 1).

The abundance frequency ratio (A/F) of each tree and shrub were > 0.05 showing a clumped or contagious pattern of distribution for each species (Table). None of the species showed regular and random patterns of distribution (Tables 1 and 2).

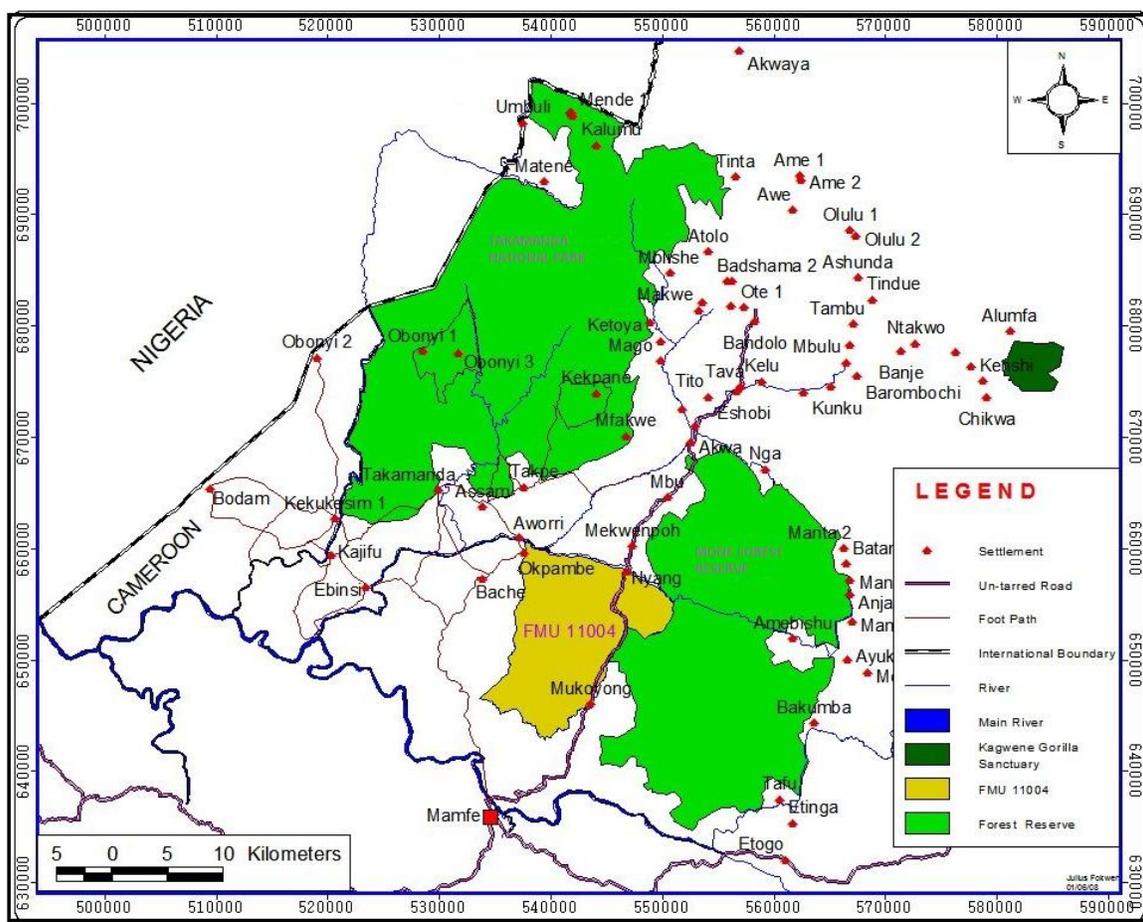


Figure 1. Map of the study area.

Shrub species composition

Twenty-seven (27) species of shrubs were recorded in the study area (Table 2). Most of the shrub species (9) belonged to the family Rubiaceae (Table 2). This was followed by the family Papilionaceae (4 species), Polygalaceae, Sapindaceae, Icacnaceae, Euphorbiaceae, Apocyanaceae and Violaceae (2 species each) and Dracaenaceae, Tiliaceae and Menispermaceae (1 species each) (Table 2). Stem density for shrub ranged from 1.56-192.19 individual ha^{-1} in the study area (Table 2). The total shrub density recorded was 770.31 individuals ha^{-1} (Table 2) with *Angylocalyx pynatii* being the most prevalent with a stem density of 148.44 individual ha^{-1} while the lowest stem density of 1.56 individual ha^{-1} was noted for the following species: *Maesobotrya staudtii*, *Penianthus camerounensis*, *Rothmanniasp.* and *Voacanga africana* (Table 2).

The basal area of shrub species varied from 1.55-307.49 $\text{cm}^2 \text{ha}^{-1}$ for the different species. The maximum

basal area of 307.49 $\text{cm}^2 \text{ha}^{-1}$ was observed for *Chytranthus macrobotrys* while the minimum basal area of 1.55 $\text{cm}^2 \text{ha}^{-1}$ was observed for *Dracaena camerooniana* (Table 2).

Among the shrubs, *Chytranthus macrobotrys* was the dominant species with the maximum IVI of 45.05 while the minimum IVI 1.24 was obtained for *V. africana* (Table 2).

Size class distribution of species

The class size distribution was recorded for trees with a girth ≥ 15 cm (Figure 1). The class size distribution of trees showed a reverse J-shaped distribution with decreasing density and with increase in girth (Figure 2). Most of the individual trees have girths from 15 – 30 cm (99 species) while 9 species showed a girth > 75 cm (Figure 2).

Shrub density at different girth classes equally showed a reverse J-shaped distribution but with a slight increase in girth classes ≥ 40 (Figure 3). The girth classes 1-10 showed the highest number of individuals while the girth class 30 to 40 showed the least number of individual per

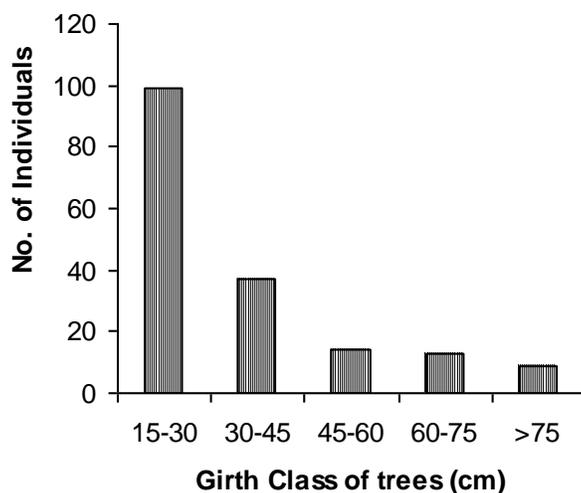


Figure 2. Girth class distribution of tree species in the study area.

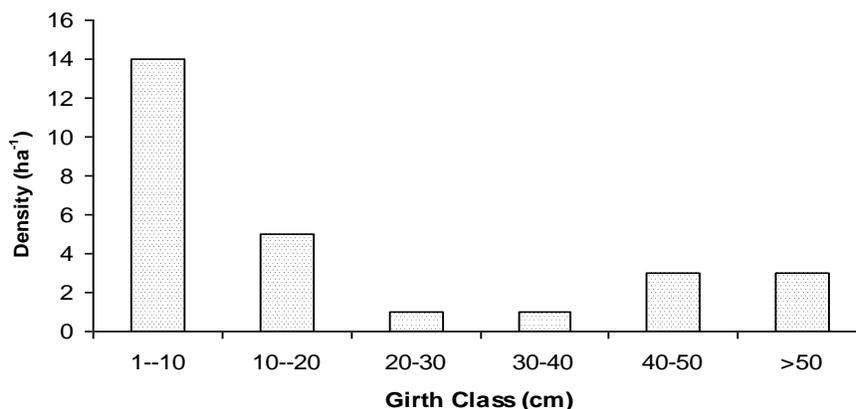


Figure 3. Density of shrubs at different girth classes in the study area.

hectare (Figure 3).

Species diversity

The Shannon – wiener's diversity index of 3.87 and 2.88 were recorded for trees and shrubs respectively (Table 3). The Simpson's dominance for tree species was 0.03 and 0.08 for shrubs (Table 3). Species evenness for trees was 0.90 and 0.87 was for shrubs (Table 3).

DISCUSSION

The Takamanda rainforest is a biodiversity conservation unit typified for its richness, endemism in flora and fauna (Ndah et al., 2012, 2013a, b; Sunderland et al., 2003). The

richness in biodiversity makes it a gene bank for most species.

The flora of the Takamanda rainforest is characterized by a variety of tree species. The Caesalpinaceae were observed to be the most prevalent family. This may be due to their fast germination ability, associated with symbiotic properties which have enabled species to easily establish within habitat types. This finding was in line with the works of Deka et al. (2012), on vegetative assessment of tree species and shrubs indicating that legumes were the prominent species recorded in the study area. Moraceae, Meliaceae and Papilionaceae also their ability to produce numerous seeds which was eventually establish at suitable sites. This result was confirmed by Khan et al. (1986) while working on regeneration and survival of

Table 3. Characteristics of trees and shrubs in the study area.

Parameter	Tree	Shrub
Shannon's diversity index (H')	3.87	2.88
Pielou evenness index (J_{sw})	0.90	0.87
Simpson index (Cd)	0.03	0.08
Richness (total number of species)	72	27

tree seedlings in tropical forests. The reasons for the low number of species observed in some families could be attributed to diseases and browsing by herbivores which resulted in poor growth and establishment and perhaps seeds need scarification treatment before germination. Similar results were reported by Coley and Barone (1996) on herbivory and plant defenses on herbivores. The low number of species could also be attributed to anthropogenic activities which affected species growth and production. Similar findings have been reported by Sumina (1994) on plant communities on anthropogenically disturbed sites in Chukotka Peninsula.

In the shrub layer, the Rubiaceae was the most dominant family in the site. The dominance of this family could be as a result of habitat adaptation and favourable environmental conditions which encourage pollination, dispersal and eventual establishment of species. Similar situations were reported by Pausas and Austin (2001) on species richness in relation to environment. Austin et al. (1996) found that edaphic parameter (soil nutrients) played a major role in species richness and establishment in an ecosystem. The reasons for the poor establishment of some families which showed lowest species may be attributed to competition for nutrients, limited light by canopy trees and destruction of undergrowth during tree snapped and logged on the forest floor. Egbe et al. (2012) mentioned similar reports in a disturbed and natural regeneration forest in Korup National Park and Coley and Barone (1996) also recorded anthropogenic activities affecting growth and distribution of species.

The stem density of tree species decreased with increase in girth class distribution in the study area. This could result from anthropogenic activities such as the logging of trees for timber, felling of trees for construction and deforestation of forest for plantation crops. This finding is in accordance with the works of Deka et al. (2012) with tree species having high stem densities at lower girth classes.

For shrubs, the highest stem density was smaller as compared to stem density of trees. The low stem densities for shrubs may be as a result of canopy trees which shade most shrubs from sunlight, thus limiting the production of photosynthates for anabolic and catabolic processes. Furthermore, poor root establishment in the soil

for nutrient and water uptake which are essential for the increase in girth and linear growth of plants.

The basal areas of tree species were comparatively higher than those of the shrubs due to the presence of large number of young individuals as well as old individuals in the community when compared with shrub species. The higher basal areas of tree species may also be due to the presence of adapted root architecture to absorb nutrients for growth. This finding is similar to the works of Parthasarathy (2001) and Parthasarathy (1999) on changes in forest composition and structure working in three sites of a tropical forest. Most of the shrub species recorded lower basal areas than those of tree species. Perhaps the low basal area could be attributed to poor root establishment for the acquisition of nutrients. Chauhan et al. (2008) reported that poor growth of tree species can be attributed to poor efficiency of some species in absorbing nutrients in the ecosystem.

Generally, species diversity is one of the most important indices used to evaluate an ecosystem. A rich ecosystem with high species diversity has a large value (H') while an ecosystem with low value (H') will have a low species diversity (Sobuj and Rahman, 2011; Deka et al., 2012). The present study site had a high species diversity for both tree and shrub species. Probably, the high species diversity for trees and shrubs could be attributed to the many tributaries and streams that empty rich organic content and mineral resources utilized by the species for growth and production. Giliba et al. (2011) reported similar findings on woodland of Bereku Forest Reserve in Tanzania.

Simpson's dominance index of trees and shrubs varied greatly within the study site. The lower the index value, the lower the dominance of species (Giliba et al., 2011). Misra (1989) reported that the greater the value of index of dominance, the lower the species diversity. This report tallies with findings of this study with high species diversity of trees and shrubs with a corresponding low dominance index value for shrub and trees in the study sites.

The species evenness values for trees and shrubs showed some similarities in the study area. This study showed to an extent some variation in the distribution of species in the study area. Similar remarks were made by Sunderland et al. (2003).

The clumped pattern of distribution of species depicts natural vegetation (Venna et al., 1999). The abundance frequency ratio (A/F) for trees and shrubs was evidence that the area was natural vegetation in which most seedlings were adapted to grow close to the mother plant. These observations were also reported by Deka et al. (2012), Giliba et al. (2011), Sobuj and Rahman (2011), Al-Amin et al. (2004) and Sugar et al. (2004) who mentioned different vegetation types.

Conclusion

This study revealed that the Takamanda Rainforest has high species (shrubs and trees) diversity. Families noted with dominant species in the study area included: trees (Caesalpinaceae, Moraceae, Meliaceae and Papilionaceae) and shrubs (Rubiaceae and Papilionaceae). However, species richness for some timber species and non timber forest products such as *Milicia excelsa*, *Azelia africana*, *Carpolobia alba* and *Annikia chlorantha* were very poor due to over harvesting for the market and livelihood support. Nevertheless, the presence of many species in the lower girth classes gives the rainforest the potential for regeneration. This indicated that effective conservation and sustainable management of the forest would make it possible for the said forest to continue providing goods and services necessary for communities around the rainforest.

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Full Length Research Paper

Effect of salt stress on osmolyte accumulation in two groundnut cultivars (*Arachis hypogaea* L.) with contrasting salt tolerance

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The effects of salt stress on the level of osmolyte accumulation in two different cultivars (K-134 and JL-24) of groundnut seedlings were studied. Seeds were grown at different concentrations of NaCl stress: 50, 100 and 150 mM and their respective controls (0.0% NaCl) for nine days. Salt stress resulted in a significant modification in the level of osmolyte accumulation in the two cultivars of groundnut. The accumulation level of osmolytes such as proline, glycine betaine, soluble sugars, free amino acids and polyamines were increased significantly in both cultivars with increasing stress severity and duration when compared with their controls. However, the percent increase of osmolyte accumulation was higher in cv. K-134 and lower in cv. JL-24. The present study indicated that cv. K-134 is salt tolerant than cv. JL-24 based on osmolyte accumulation and growth parameters. The osmolyte accumulation in relation to the salt tolerance of these cultivars was discussed.

Key words: Osmolytes, salt tolerance, groundnut, cultivar.

INTRODUCTION

Soil salinity is one of the most significant agricultural problems in arid and semi-arid regions in different parts of the world. Nearly 20 to 40% of the world's cultivated area and half of the world's irrigated land are affected by salinity (Rhodes and Loveday, 1990; Flowers, 2004). The economic prosperity of a nation like India, where a majority of population is primarily dependent on agriculture depends on crop productivity. Soil salinity creates extremely unfavorable conditions for plant growth and development. In response to various environmental stresses such as salt and drought stresses, plants have developed different physiological and biochemical mechanisms to adapt or to tolerate stress (Bartels and Salamini, 2001; Rahnama and Ebrahimzadeh, 2004; Faical et al., 2009). An understanding of the mechanism of plant salt tolerance

will lead to effective means to breed or genetically engineer salt tolerant crops. Indeed, osmolyte accumulation in plant cells results in decrease of the cell osmotic potential and thus in maintenance of water absorption and cell turgor pressure (Blum et al., 1983). A number of investigators (Xiong and Zhu, 2002; Rahnama and Ebrahimzadeh, 2004) have reported that the osmotic adjustment in plants subjected to salt stress occurs by the accumulation of high concentrations of osmotically active compounds known as osmolytes such as proline, glycine betaine, soluble sugars, free amino acids, polyamines, etc, in order to lower the osmotic potential.

Osmoprotectants serve to raise osmotic pressure in the cytoplasm and can also stabilize proteins and membranes when salt levels or temperatures are unfavorable,

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therefore osmoprotectants can play an important role in the adaptation of cells to various adverse environmental conditions (McNeil et al., 1999; Jagesh et al., 2010).

Proline accumulation was found to be an early response to salt stress, which acts as an osmotic protectant and increased accumulation shows greater tolerance to salt and drought stress (Fedina et al., 2002). Increased levels of proline contribute to the turgor maintenance of cells and its accumulation is considered as a stress indicator in several plant species under salt stress conditions (Giridarakumar et al., 2003; Jagesh et al., 2010). Glycine betaine is one of the quaternary ammonium compounds and is regarded as an effective compatible solute that accumulates in the chloroplasts of certain plants when exposed to environmental stresses, such as drought and salinity. It could play a major role in maintaining intracellular osmotic equilibrium during stress conditions (Subbarao et al., 2001; Giridarakumar et al., 2003). The accumulation of soluble sugars in plants has been responsible for salinity or drought stress and act as osmoprotectants (Murakeozy et al., 2003). According to Cram (1976), sugars contribute up to 50% of the total osmotic potential in glycophytes subjected to the saline conditions. Further, the amino acid accumulation associated with stress may actually be a part of an adaptive process contributing to osmotic adjustment and increased level of free amino acids together with organic acid and ammonium compounds serve as compatible cytoplasmic solutes to maintain the osmotic balance under stress conditions (Dubey, 1994).

Furthermore, the increase in total polyamine content in plants has been shown to occur in a variety of plant species in response to salt stress (Pedro et al., 2004). Polyamines are cationic molecules, positively charged under intracellular pH, which are essential for plant growth and differentiation. It has been reported that polyamines in plants protect plasma membrane under salinity stress and thus enhance salt tolerance (Mansour and Al-Mutawa, 1999).

Groundnut (*Arachis hypogaea* L.) is an important oilseed cash crop for all tropical and sub-tropical regions of the world. Anantapur, a district in Andhra Pradesh, India, occupies the first place in groundnut cultivation with 1.88 million hectares and the production of about 1.2 million tones (www.icrisat.org). Information is lacking regarding the relative levels of salt tolerance among the existing groundnut cultivars. Hence, the present study was aimed to make comparative analysis of tolerance potentials based on osmolyte accumulation in two different groundnut cultivars differing in salt tolerance. Further, these lines are used in improving programmes; it seems to be effective and economic improvement.

MATERIALS AND METHODS

Seeds of groundnut (*A. hypogaea* L.) cultivars namely (cv. K-134 and cv. JL-24) were procured from Acharya N.G.Ranga Regional research station, Kadiri, Andhra Pradesh, India. Seeds were surface sterilized with 0.1% (w/v) mercuric chloride for 5 min and tho-

roughly rinsed with distilled water and allowed to germinate in Petri dishes of 15 cm diameter lined with filter papers. Distilled water alone served as control, while for stress treatment, NaCl solution was added at concentrations of 50, 100 and 150 mM and kept in dark at $24\pm 1^\circ\text{C}$ under aseptic conditions for three days. After three days the Petri dishes were transferred to a programmed plant growth chamber under controlled conditions (light/dark regime of 16/8 h at $24\pm 1^\circ\text{C}$, relative humidity of 60-65%, photon flux density $350\ \mu\text{mol m}^{-2}\ \text{s}^{-1}$) up to nine days. After nine days total seedlings were used for experimental analysis.

Free proline content was extracted from both control and stressed groundnut seedlings in 3% aqueous sulfosalicylic acid and estimated by Bates et al. (1973) using ninhydrin reagent. Quaternary ammonium compounds were extracted and measured as glycine betaine from 0.5 g of dried material, according to Grieve and Grattan (1983). Soluble sugars were extracted following the method of Highkin and Frankel (1962) from 0.5 g of dried tissues in 80% ethanol by heating at 80°C for 30 min and centrifuged, then supernatant was used for the estimation of total soluble sugars following Nelson (1944) method as modified by Somogyi (1952). The oxidation of sugars by copper was monitored at 500 nm by formation of colorimetric complex of arsenic and molybdenum salts, using UV-visible spectrophotometer (helios- α thermospectronic).

The extraction and estimation of amino acids were determined spectrophotometrically according to Moore and Stein (1948). The extraction and estimation of total polyamine content was done according to Seiler and Wiechmann (1967). 0.5 g of plant material was homogenized with 5 ml of 5% perchloric acid (HClO_4), centrifuged at 12000 rpm for 20 min. Supernatant fraction was dansylated, and then benzene extract was separated on TLC plates coated with silica gel by using chloroform : triethylamine (25:2 v/v) as solvent system. Polyamines were identified with the help of authentic samples. The spots were eluted with ethyl acetate and quantified using a UV-visible spectrophotometer (Thermo- Spectronic, USA).

RESULTS

Seedling growth

The growth of the seedlings (shoot and root length) was measured in both control and stressed conditions on day 9 (Figure 1). The total seedling growth was decreased in both cultivars during salt stress. However, the inhibition of seedling growth was found to relatively less in cv. K-134 than cv. JL-24 during severe stress treatment (150 mM NaCl).

Free proline content

Free proline content was estimated in control and NaCl stressed seedlings of two groundnut cultivars and data are presented in Figure 2. The free proline content was significantly increased in stressed plants over control plants. Nevertheless, a significant difference was found in free proline accumulation between the cultivars by about 3.0 and 2.6 fold in cv. K-134 and cv. JL-24 respectively at 150 mM NaCl stress when compared to their respective controls.

However, the percent increase was comparatively more in cv. K-134 than in cv. JL-24.

Glycine betaine

The level of glycine betaine content was significantly increa-

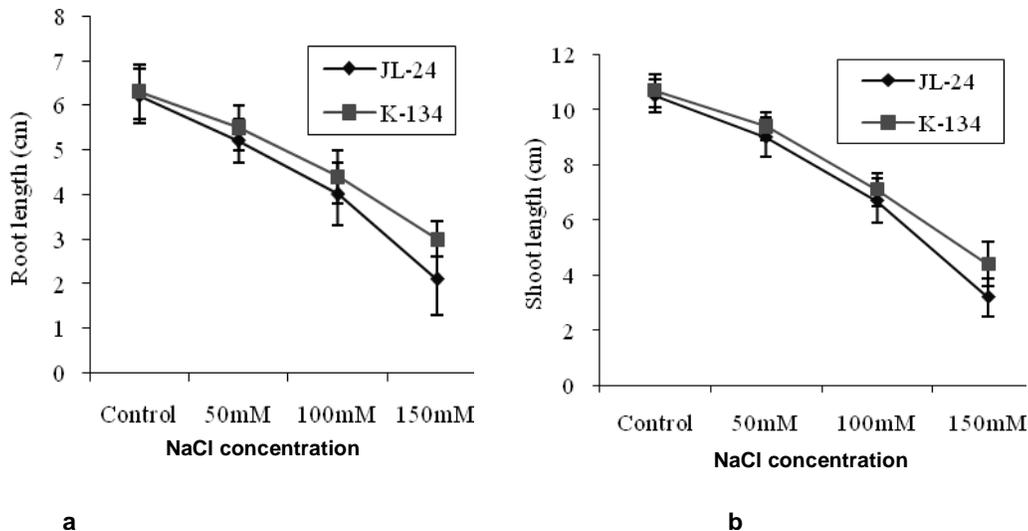


Figure 1. Root length and shoot length in two cultivars of groundnut under control and NaCl stress. (a) Root length (cm); (b) shoot length (cm). Values are mean of five replications. Vertical bars indicate \pm S.D.

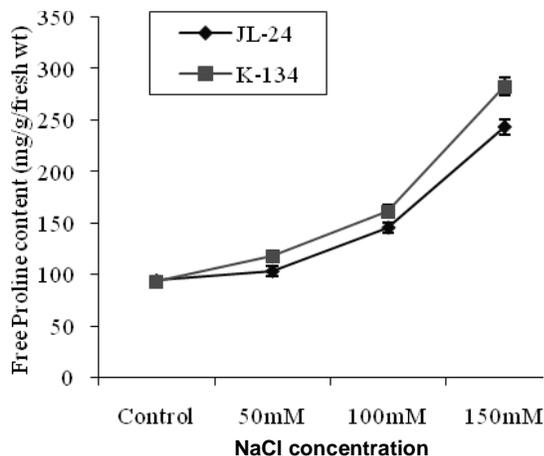


Figure 2. Free proline content in two cultivars of groundnut under control and NaCl stress. Values are mean of five replications. Vertical bars indicate \pm S.D.

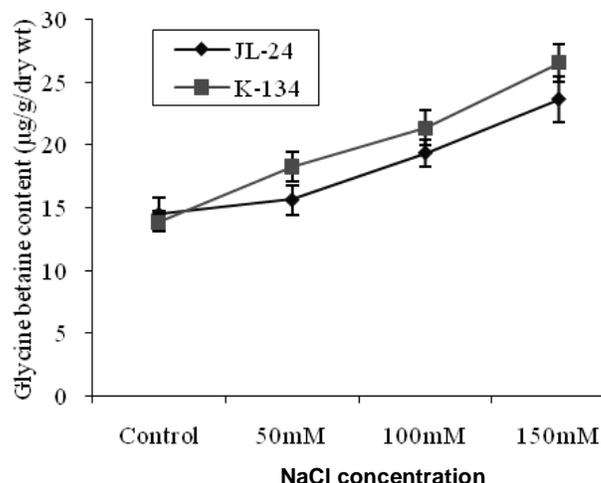


Figure 3. Levels of quaternary ammonium compounds (glycine betaine equivalents) in two cultivars of groundnut under control and NaCl stress. Values are mean from five replications. Vertical bars indicate \pm S.D.

sed in both cultivars at all stress regimes. However, the rate of increase was significantly different in both cultivars (Figure 3). The rate of increase in glycine betaine content was found to be higher in cv. K-134 than cv. JL-24 at severe stress level (150 mM NaCl).

Soluble sugars

Total soluble sugar content was increased with increasing severity of stress in both cultivars of groundnut (Figure 4). However, the degree of increase in soluble sugar content was dependent on species tolerant potential and stress severity. There was, by about 3.3 fold

increase in cv. K-134, 2.5 fold increase in cv. JL-24 at 150 mM NaCl stress when compared with their respective controls.

Total amino acids

The pool sizes of amino acid levels were increased significantly in both cultivars at all stress regimes (Figure 5). However, the degree of increase in free amino acid contents was more in cv. K-134 than cv. JL-24. Therefore, the accumulation of total amino acids showed increase by

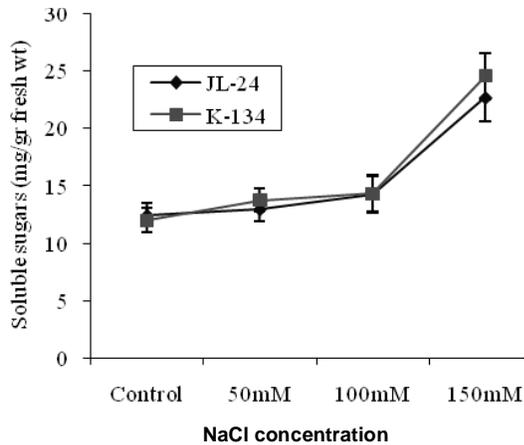


Figure 4. Levels of soluble sugars in two cultivars of groundnut under control and NaCl stress. Values are mean of five replications. Vertical bars indicate \pm S.D.

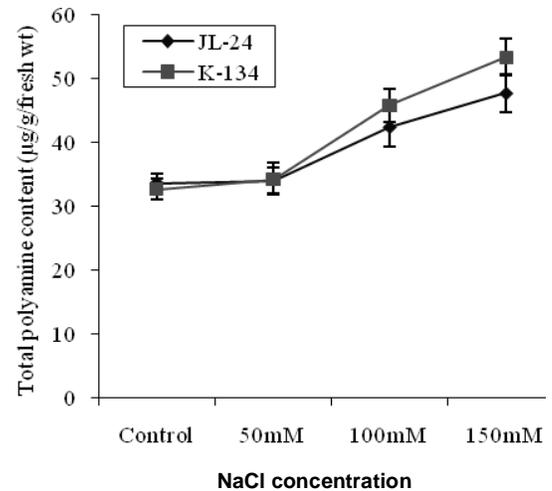


Figure 6. Total polyamine content in two cultivars of groundnut under control and NaCl stress. Values are mean of five replications. Vertical bars indicate \pm S.D.

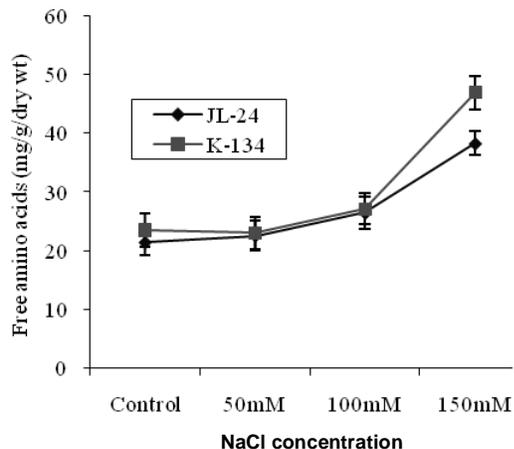


Figure 5. Total amino acid content in two cultivars of groundnut under control and NaCl stress. Values are mean of five replications. Vertical bars indicate \pm S.D.

about 2.1 fold in cv. K-134, 1.8 fold in cv. JL-24 at severe stress (150 mM NaCl).

Total polyamines

Total polyamine content was increased significantly with severity of stress in all cultivars (Figure 6). Nevertheless, a difference in the accumulation of total polyamine content was observed between the cultivars. However, the percent increase was found to be higher in cv. K-134 than in cv. JL-24.

DISCUSSION

There is a general agreement that the whole plant growth responses to salinity is multigenic and that a better know-

ledge of the underlying physiology is required in order to understand why some plant species and varieties are more salt resistant than others. This is a complex task since plant growth responses to salinity can vary with degree and duration of stress, plant organ, variety or species and developmental stage (Neumann, 1997). It has been shown that the stress caused by salts present in the soil alters water status and brings about initial growth reduction of the plant (Yeo, 1998; Fatemeh et al., 2010). One of the classic manifestations of salt stress in many plant species is marked reduction in plant height, due to the osmotic effects of the salt outside the roots which distinguishes a salt-susceptible plant from a more tolerant one (Munns et al., 1995). Similarly, in the present study, we recorded reduced growth during stress conditions and degree of reduction in seedling growth was dependent on intensity or severity of stress. It revealed that the 150 mM NaCl stress treatment has caused significant reduction in both cultivars, but more pronounced reduction was found in cv. JL-24 than cv. K-134 (Figure 1). Several investigators (Mishra et al., 1996; Fatemeh et al., 2010) reported reduced growth in different plant species under salt stress. This reduced growth under salinity stress has been ascribed either to osmotic or ionic effects; inhibition of cell division and cell elongation process associated with the growth of the seedling and decrease in plastic extensibility of the growing cell walls.

The accumulation of compatible solutes may help to maintain the relatively high water content obligatory for plant growth and cellular functions. Osmotic and oxidative stress induced by salinity could be reduced by the production and accumulation of compatible solutes. Osmoprotectants and their accumulation play a key mechanism in the plants for increasing yield of crops subjected to stress conditions. The levels of osmoprotectants increased during exposure to stresses such as salinity, water deficit,

and low temperature (McNeil, 1999). The frequently observed metabolites with an osmolyte function are proline, glycine betaine, soluble sugars, free amino acids and polyamines.

The accumulation of proline is an early response to salt stress (Fedina et al., 2002). Several investigators (Delauney and Verma, 1993; Kavikishor et al., 1995) have demonstrated that the positive correlation was found between the accumulation of proline and osmoprotective role at the whole plant level and cell cultures. Convincing evidence is still lacking as to whether accumulation of proline can provide any biochemical adaptation for plants during stress. Giridarakumar et al. (2003) in mulberry, Veeranagamallaiah et al. (2007) in foxtail millet and Fatemeh et al. (2010) in potato demonstrated the differences in proline accumulation and a positive correlation between magnitude of free proline accumulation during salt and water stress. Similarly, in the present study, we have noticed a positive correlation between salt stress and free proline accumulation between the two groundnut cultivars (Figure 2), however a greater accumulation rate of free proline content (3.7 fold) was found in salt tolerant cv. K-134, where as the salt sensitive cv. JL-24 showed lesser accumulation rate (2.0 fold). The result obtained in this study further strongly supports Fatemeh et al. (2010) who reported the increased accumulation of in tolerant potato variety. Free proline accumulation and salt tolerance has been suggested as an index for determining salt tolerance potentials between the cultivars (Sudhakar et al., 1993; Giridarakumar et al., 2003; Veeranagamallaiah et al., 2007). In contrary, very few reports for instance Lutts et al. (1999) reported that salt sensitive cultivars accumulated significantly higher levels of proline accumulation compared to the tolerant ones.

Glycine betaine is regarded as an effective compatible solute that accumulates in the chloroplast of plants, when exposed to environmental stresses (Sawahel, 2004). Here, we reported on a positive correlation between glycine betaine accumulation and salt stress and also observed a genotypic variation in glycine betaine accumulation in two groundnut cultivars (Figure 3). An increased accumulation of glycine betaine content was noticed in tolerant cv. K-134 than cv. JL-24. Parallel to these results, an increase in glycine betaine content with increasing salt stress was found in green gram and mulberry (Sudhakar et al., 1993; Giridarakumar et al., 2003).

Several investigators have noticed that accumulation of glycine betaine under salt stress was found to be high in salt tolerant species (Jagendorf and Takabe, 2001). Besides osmoregulation glycine betaine stabilizes the oxygen evolving activity of photosystem-II protein complexes at high concentration of NaCl. The major role of glycine betaine might be to protect membranes and macromolecules from damaging effects of stress (Sawahel, 2004).

Soluble sugars have been specified as potential osmoregulators (Raggi, 1994). Elevated sugar levels relative to

control in salt stressed plants may contribute to the turgor maintenance (Sacher and Staples, 1985). In the present study, the amount of elevated soluble sugars was relatively higher in tolerant cv. K-134 and lesser in salt sensitive cv. JL-24 at severe stress treatments (Figure 4). In analogy, several investigators noticed that soluble sugar levels were increased with increased level of salt stress, (Dubey and Singh, 1999; Murakeozy et al., 2003). Furthermore, Jouve et al. (2004) observed a higher accumulation of soluble sugars in aspen at 150 mM NaCl stress.

The changes in accumulation of free amino acid content induced by salt stress have an important role, since these relations were obtained several times in a relationship with stress tolerance by Livia et al. (2002) in cereal plants. Survival and growth of plants in saline environments is the result of adaptive processes such as ion transport and compartmentation of osmotic solute, synthesis and their accumulation lead to the osmotic adjustment and protein turnover for cellular repair (Munns and Termaat, 1986). In the present study, we have noticed the existence of variation in the accumulation of amino acid levels among the cultivars studied; the extent of increase was greater in the tolerant cv. K-134 than salt sensitive cv. JL-24 (Figure 5). Similar results were obtained by Livia et al. (2002) in cereal plants and Ramanjulu and Sudhakar (1997) in mulberry. Varietal variations in the magnitude of accumulation of amino acids have been taken as an index for determining the salt tolerant potentials of many crops (Madhusudan et al., 2002). Improved levels of free amino acids together with organic acids and quaternary ammonium compounds serve as compatible cytoplasmic solutes to maintain the osmotic balance under stress conditions (Dubey, 1994).

Polyamines are known to be involved in various cellular processes (Rajam et al., 1998) and they are ubiquitous aliphatic amines that are implicated in many aspects of plant growth and developments in a wide range, and play an important role in stabilizing the plasma membrane under salt stress condition (Galston and Sawhney, 1995). Effect of salt stress on polyamine metabolism is not always clear, since differences in polyamine accumulation in response to salt stress have been reported among and within the species (Pedro et al., 2004). Here, we noticed a positive correlation between salt tolerance and accumulation of higher levels of polyamines and exhibited genotypic variation (Figure 5). However, a greater accumulation was found in tolerant cv. K-134 than salt sensitive cv. JL-24. Similarly, Chattopadhyaya et al. (2002) and Fatemeh et al. (2010) noticed a higher accumulation of polyamine content with varying levels in seven different plant species under salinity stress.

From this study, it is clear that cv. K-134 shows better salt tolerant nature as compared to cv. JL-24 based on above results through physiological and biochemical marker traits. Interesting features found through these results must be related to salt stress response and should be considered as general salt stress reaction markers for

groundnut. Moreover, further independent analysis of molecular level may help in understanding the salt tolerant potentials of groundnut cultivars for breeding programmes in future.

ACKNOWLEDGEMENT

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Full Length Research Paper

The effects of pH and growing medium type on the susceptibility of *Moringa oleifera* to fungal diseases during seedling emergence

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Moringa oleifera seed germinates poorly and most seedlings die during early establishment. To solve this problem, the effects of pH and growing medium type on the susceptibility of *M. oleifera* to fungal diseases during seed emergence and early seedling establishment were evaluated in a 3 x 6 factorial experiment arranged as a randomized complete block design with three replications in a greenhouse experiment. Six growing medium types (sandy soil, clay soil, pine bark, clay + sandy soil, clay + pine bark and sand + pine bark) were evaluated at pH 6.2, 8.2 and *in situ* pH for each growing medium type. The control was the sterilised media of *in situ* pH. Seed viability tests showed 90% viability. Analysis of the seed showed the presence of *Fusarium* spp., *Pythium* spp., *Dreschlera* spp., *Rhizopus* spp. and *Chaetomium* spp. Thus, seed was sterilised prior to planting to eliminate any pathogen from the surface of the seed. Media and pH were found to have a significant effect on the emergence of seeds. *In situ*, sterilised pine bark and clay gave the highest emergence. After emergence, no seedlings showed any infection indicating that reported seedling deaths could be a result of seed borne diseases. Observations indicated *Fusarium* spp. infection in the seed that had failed to germinate, whilst those in the sterilised media showed no evidence of fungal attack. This means media used for *Moringa* ought to be sterilised with hypochlorite or another material or procedure to ensure good seed emergence.

Key words: pH, susceptibility, viability, media, pathogens, emergence, sterilised, *Fusarium* spp.

INTRODUCTION

Moringa belongs to the family Moringaceae, a genus comprising of thirteen tree species that grow in the tropical and subtropical regions of the world. *Moringa oleifera* is a soft wood native to India with great potential in combating extreme hunger and poverty. These nutritious trees grow quickly in many environments and can feed people as well as livestock. Nutritional analyses has shown that *Moringa* leaves contain large amounts of several important nutrients such as vitamin A and C, calcium, potassium and surprisingly contains all essential amino acids and complete proteins, which is rare for a plant (Anonymous, n.d.).

The use of *Moringa* in Zimbabwe has been largely medicinal, especially in the management of HIV/AIDS (Monera and Maponga, 2011). This is not surprising because *Moringa* has been reported to have numerous clinical benefits (Fahey, 2005). In addition to this, *Moringa* is now an essential part of many individual nutritional gardens in Zimbabwe, though there is no commercial production. *Moringa* is a proven water purifier with remarkable nutritional value and happens to grow in places where bad water, poor diets and diseases cause high mortalities (Fritz, 2000).

Pilot studies have been done to determine the effects

of different medium types, pH levels and fertilizer types on the general growth of the tree. In these studies as well as in some nurseries, it was also observed that a significant proportion of the seedlings succumbed to fungal diseases (Chimonyo, 2006; Goss, 2007). This can be a major setback in *Moringa* nurseries, and can result in reduced quantity and quality of *Moringa* seedlings. In spite of this, *Moringa* has received minimum attention. This is more so in relation to studies on diseases that affect the tree at various stages of development. At germination, it is likely that medium type and pH could be important.

Soils and soil conditions affect the growth of crops indirectly by their effect on disease, among many factors, whilst adverse soil conditions such as poor drainage greatly increase the chances of serious infection with root fungi (Davies et al., 1993). Soil pH affects pathogen development in the soil since soil is a natural reservoir of inoculum.

A gram of soil normally contains ten to a hundred meters of mycelia fragments (Foth, 1990). Fungi cause a very wide range of disease in plants. Many soil borne fungal plant pathogens cause diseases of the roots or stems, thus disrupting the uptake and translocation of water and nutrients from the soil. This may result in appearance of symptoms similar to drought and nutrient deficiencies, which include wilting, yellowing, stunted growth and plant death. The fungi, which commonly cause seedling death, include *Pythium* spp., *Phytophthora* spp., *Rhizoctonia* spp., *Sclerotium* spp. and *Fusarium* spp. (Agrios, 1988). Reports of diseases that affect *Moringa* after emergence are few (Mandhokhot et al., 1994).

A disease caused by *Drechslera haraiensis*, whose major symptom is the extensive rotting of pods has been reported (Rajangam et al., 2001). Zimbabwean *Moringa* growers indicate that there are cases of diseases similar to fungal wilts and damping off in a significant number of *Moringa* nurseries. However, no research has been done to confirm or refute these claims.

MATERIALS AND METHODS

Study site

The experiment was carried out at the University of Zimbabwe, Crop Science Department in a greenhouse.

Experimental design

The experiment was laid out as a 3 x 6 factorial, arranged in a randomized complete block design. Factor 1 was pH with 3 levels namely: *in situ* pH, pH 6.2 and 8.2 on the calcium chloride scale. Factor 2 was growing medium type with six levels namely: clay, sand, pine bark, sand + pine bark, clay + sand and clay + pine bark. Each treatment was replicated once in each of three blocks. The control was the *in situ* pH and was sterilised for each growing medium type.

pH amendment

The pH of each media was determined and the pH amended to the desired level using lime to increase pH and ferrous sulphate to lower pH. After amendments, the media were left to stabilize for about three weeks after which measurements were done to check whether the required pH levels had been obtained. The *in situ* pH of the respective media were as follows: clay pH 6.2, pine bark pH 4.2, sand pH 5.8, sand + pine bark pH 5.6, clay + pine bark pH 6.0 and 5.9 for clay + sand pH 5.9.

Seed tests, pathogen analysis and planting

Viability was tested prior to planting and the seed lots were found to have 90% viability. Laboratory analysis was done to examine seeds for seed borne pathogens before sowing and the following pathogens: *Rhizoctonia* spp., *Rhizopus* spp., *Pythium* spp., *Chaetomium* spp. and *Drechslera* spp. were identified. *Fusarium* spp. attacked 30% of the seedlings, *Chaetomium* 10%, and *Rhizopus* spp. 12%. The seeds were then washed with sterile water and dipped in a 1:6 solution of hypochlorite and water. Thereafter, the seeds were incubated at a temperature of 26°C for ten days. The seeds were lastly immersed in hot water at 75°C for five minutes before planting.

Five seeds were planted in each polythene bag of 25 cm diameter. Each seed was sowed at a depth of 1 cm. Watering was done prior to planting the seed and 0.3 L of water was applied in each growing medium type twice a week.

Data collection and analysis

Disease severity and incidence were measured. Severity of any fungal disease was measured on a scale of 1 - 5, with 5 being the most severely infected count and 1 being the least infected. Incidence was obtained by counting the number of diseased plants. Assessment of the media was done to determine the presence of naturally occurring fungi in each growing medium, within each media using water based agar and microscopy for identification. In addition to this, slides were prepared using the tissue from diseased plants and seeds which had not germinated.

Results were analyzed using Genstat and Analysis of Variance (ANOVA) was carried out for emergence of seeds and disease incidence and severity. Fisher's Protected Least Significance Difference test (LSD) at 5% was used to separate means.

RESULTS

Effects of pH and medium type on emergence

Clay

The pH of 6.2 produced the least germination, which was significantly lower ($P < 0.05$), than that of pH 8.2 or the *in situ* sterilised clay. The two, pH 8.2 and the sterile *in situ* clay, were not significantly different and both had high germination (Figure 1). The results were somewhat reversed in the case of the sand. Here, pH 6.2 and 8.2 significantly ($P < 0.05$) increased germination when compared with the sterile *in situ* sand. The sterile *in situ* sand had the lowest germination (Figure 1).

Germination from pine bark was not affected by pH

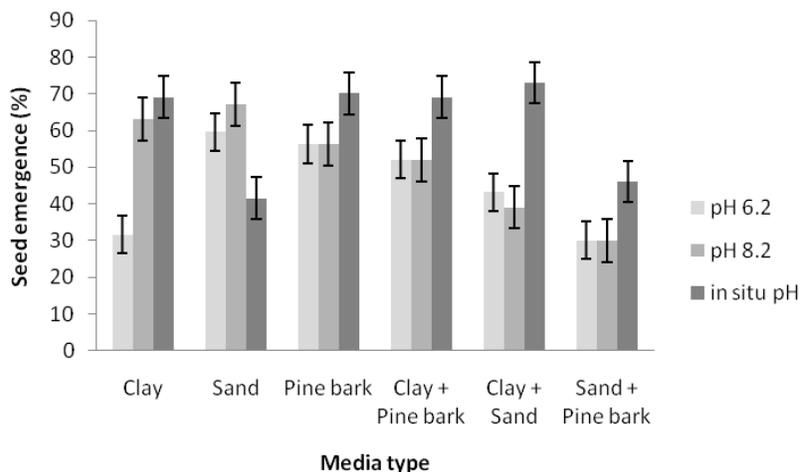


Figure 1. Effects of pH and growing medium type on seedling emergence. Error bars indicate LSD at 5%.

although the sterile pine bark tended to increase germination. Similar results were observed with the mixture of pine bark and clay, showing, perhaps the dominant pine bark effect (Figure 1). A similar trend was observed when pine bark was mixed with sand although this mixture produced very low germination. On the other hand, the clay and sand mixture was somewhat additive in the case of the sterile mixture but antagonistic with the other treatments.

In summary, the germination of *Moringa* was affected differently by different media but tended to be higher with the sterile media in all growing medium types except when pine bark was mixed with sand or when sand was alone.

Effect of growing medium type and pH on fungal infection

There was no incidence of fungal disease on the seedlings in the nursery after emergence during the duration of the experiment. The seeds which failed to germinate were dug up and analyzed for fungal infection. Identification procedures in the laboratory indicated the pathogen which had caused the rotting to be *Fusarium* spp. with no other fungi being present. However, the seed from the sterilised media showed no signs of infection upon analysis. The degree or severity of seed rotting however varied from treatment to treatment as indicated by the variation in colour and level of rotting observed in the seeds being analyzed (Table 1).

DISCUSSION

Seed viability tests carried out prior to planting indicated that the seed used had a viability of 90%. Growing medium type had a significant effect on emergence,

affecting the rate of emergence, with all media except sand exhibiting high germination in the *in situ* pH growing medium. The sand at pH 8.2 showed the most pH-dependent improvement over the *in situ* treatment. These results are supportive of the preliminary studies done (Goss, 2007) where the largest *Moringa* biomass production was realized in the sandy soil and these studies further indicated that *Moringa* performed well in pH ranges of 7.6 to 8.7 (calcium chloride scale).

This study shows that the use of good quality seed and sterilisation of media may solve the poor germination of *Moringa*. However, soil borne fungal pathogens which infect seeds and roots are a serious constraint to nursery production as they affect seedling establishment leading to poor emergence and delayed development of the seedlings. There was no fungal infection associated with the pine bark media. This commercial material must therefore have been free from disease pathogen contamination. Use of composted pine bark in planting media has been reported to result in successful control of diseases caused by several soil borne fungi such as *Pythium* spp., *Phytophthora* spp. and *Rhizoctonia* spp. (Agrios 1988).

Laboratory seed analysis revealed that the seeds which failed to germinate had been infected by *Fusarium* spp. This is an interesting finding. It appears that despite reports that *Moringa* is highly resistant to diseases, it is in fact susceptible to *Fusarium* spp. resulting in seed rots and reduced germination. In this experiment, analysis of the seed which had failed to germinate suggested that poor germination may have been a result of seed borne diseases or soil borne pathogens. Whilst findings from this study are supportive of preliminary studies (Goss, 2007) which indicated high susceptibility of *Moringa* seed to fungal infections, there is need for further studies. Such studies could use artificial infection.

The poor germination, which was associated with the *in situ* sterilized sand, suggested that other factors affected

Table 1. Analysis of seed that failed to emergence for pathogen infection.

Treatment	Color observed	Type of fungi identified
Clay ^{1*}	Brownish	<i>Fusarium</i> spp.
Clay ²	Creamish brown	<i>Fusarium</i> spp.
Sand ¹	Creamish brown	<i>Fusarium</i> spp.
Sand ²	Brownish black	<i>Fusarium</i> spp.
Pinebark ¹	Brown	<i>Fusarium</i> spp.
Pinebark ²	Creamish brown	<i>Fusarium</i> spp.
ClayPinebark ¹	Blackish, brown	<i>Fusarium</i> spp.
ClayPinebark ²	Brownish, cream	<i>Fusarium</i> spp.
ClayaSandS ¹	Dark brown ,black	<i>Fusarium</i> spp.
ClaySandS ²	Dark brown	<i>Fusarium</i> spp.
SandPinebark ¹	Brownish	<i>Fusarium</i> spp.
SandPinebark ²	Creamish brown	<i>Fusarium</i> spp.
ClayPinebark ³	Creamish	No fungi identified
Clay ³	Creamish	<i>Fusarium</i> spp.
Sand ³	Creamish	No fungi found
Claysand ³	Creamish brownish	<i>Fusarium</i> spp.
SandPinebark ³	Creamish	No fungi found
Claysand ³	Creamish	No fungi found

*1: pH 6.2, 2: pH 8.2, 3: *in situ* pH + sterilized.

germination in this growing medium. It is conceivable that the good drainage associated with sand could have led to poor moisture availability for the germinating seed. However, despite the presence of *Fusarium* spp. in sand at pH 6.2 and 8.2, germination was good, showing perhaps that the good drainage could have somewhat led to the leaching of the pathogen.

Lastly, contrary to expectations, there were no incidences of fungal diseases in the field after emergence of the seedlings. This suggests that seed borne diseases may account for the reported poor germination of *Moringa* and not diseases coming after emergence.

Conclusion

Moringa emergence and initial seedling growth rate is influenced mainly by medium type and infection by *Fusarium* spp. which causes seed rots. Sterilisation of media appeared adequate in dealing with this pathogen. The use of a commercial disease free growing medium such as pine bark ought to be promoted. If natural soil is required, then clay, which has to be sterilised, could be used.

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Full Length Research Paper

Knowledge on cassava disease management: The case of cassava brown streak disease awareness in Northern Uganda

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The cassava brown streak disease (CBSD) has spread rapidly in Northern Uganda since its emergence in Uganda in 2005. Field surveys conducted in 2010 and 2011 by Ngetta Zonal Agricultural Research and Development Institute (Ngetta ZARDI) revealed high CBSD prevalence in the zone. CBSD epidemic has severely affected livelihood of smallholder farmers in the region. Lack of knowledge on disease recognition and management contributed significantly to rapid spread of CBSD in the zone. Addressing this aspect to increase and improve the knowledge base of cassava farmers was an important component in integrated disease management. Ngetta ZARDI through the Agricultural Technology Agribusiness and Advisory Services (ATAAS) project developed a multi-disciplinary effort to reduce the spread and impact of CBSD, partly through promotion of adoption of tolerant cassava varieties, and educating and training of smallholder farmers and other stakeholders. Participants were carefully selected from farmer groups, extension and political leadership. Training involved lectures and field visits for practical demonstration of CBSD symptoms. The training increased the number of people with knowledge on CBSD management up to farmer level. Overall, the training program significantly strengthened the human resource base employed against CBSD in the zone.

Key words: Cassava brown streak disease, Northern Uganda.

INTRODUCTION

Cassava is a major staple food for more than 200 million people in Eastern and Central Africa, most of which are living in rural areas. The crop has been prioritized by NEPAD as a "poverty fighter" (NEPAD, 2004). Cassava plays a key role as food security and income generating crop for majority of the farming communities in Northern Uganda (UBOS, 2010). Despite the importance of cassava in Northern Uganda, farm yields are about 12 t/ha in comparison to 40-50 t/ha achievable in good growth conditions (UBOS, 2012). These low yields are due to constraints that challenge the production and utilization of the

crop: pests and diseases; the use of inferior and low yielding varieties; lack of good quality planting materials; poor farm management and husbandry practices and variability in weather patterns. Among the major diseases, viral disease; CBSD and cassava mosaic disease (CMD) are the most important in the zone. CBSD is the most damaging disease causing over 60% yield loss and threatening the livelihoods of small holder farmers.

Cassava brown streak disease (CBSD) is caused by cassava brown streak virus (CBSV) (Moner et al., 2001) and Ugandan cassava brown streak virus (UCBSV)

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Figure 1. Symptom of CBSD on leaves (patches of yellow areas mixed with normal green colour, more prominent on mature lower leaves).



Figure 2. Symptoms of CBSD on stems (brown streaks on the stem).



Figure 3. CBSD symptoms in root tubers-sections of the edible part of storage root turn brown or yellow and hard.



Figure 4. Tuber constrictions due to CBSD infection, forming a bead like appearance.

which affects all parts of the plant, causing characteristic above and below ground symptoms (Alicai et al., 2007; Hillocks and Jennings, 2003). The disease affects the yield and quality of the tuberous roots of cassava (*Manihot esculenta* Crantz). The economically damaging symptoms occur on the tuberous roots in the form of yellow/brown, corky necrosis in the starch bearing tissues (Figure 3), and radial root constriction in very severe infections (Figure 4). The necrosis begins as discrete areas, but in fully susceptible cultivars, it may affect most of the root, rendering the roots unfit for human consumption (Hillocks and Jennings, 2003; Hillocks et al., 2001, Nichols, 1950). Foliar symptoms of the disease are expressed on leaves (Figure 1) and stems (Figure 2).

It has been known for some time that CBSD symptoms can be expressed at altitudes greater than 1,000 m above sea level when infected cuttings have been planted. This occurred in Uganda when infected material was taken from Tanzania in 1934, but the disease was eradicated by destroying all plants showing symptoms (Jameson, 1964). From that time until 2004, CBSD has not been prevalent in Uganda, although CBSD-like symptoms were observed on a few cassava plants at one site in central Uganda in 1994 (Thresh et al., 1994). CBSD re-emerged in Uganda in 2005 (Alicai et al., 2007). The re-emergence of CBSD in Northern Uganda raises concern for food production and household income for farmers in the Northern agro-ecological zone of Uganda who regard cassava as their major staple food. Most farmers in the zone grow CBSD susceptible varieties, resulting in high loss in yield (personal communication). Thus if not controlled, CBSD is likely to bring cassava production in the zone to a halt. CBSD has been considered one of the most dangerous diseases in the world (Donald, 2010). The overall effect of CBSD is reduction of root yield by up to 74% (Muhana et al., 2004) and quality (Hillocks et al., 2001). When combined with cassava mosaic disease,



Figure 5. CBSD sensitization workshop.

100% yield loss can result. In terms of control, the most economically viable method for cassava brown streak disease management is the host-plant resistance (Munga, 2008). To date no CBSD resistant cassava has been developed.

At the time of planning for the CBSD awareness campaign there was inadequate information with regard to the disease. Farmers, extension staff, NGOs and policy makers in northern Uganda did not know how CBSD expresses itself, its effects and how it spreads. It was therefore, very important to create awareness on the severity and effects of the disease and how to manage it, while researchers continue their efforts in discovering more effective means of overcoming the CBSD pandemic. Resource materials on CBSD that can be used in CBSD awareness campaigns were developed and used to train trainers within northern Uganda to help carry out the campaigns in the different sub-counties in the northern agro-ecological zone of Uganda.

MATERIALS AND METHODS

A training program was executed in a cascade model that progressed through three levels (sub-county, parish and village levels). The training started from sub county level (level 1) through parish level (level 2) to community/farmer level (level 3). Level 1 was initiated with a training workshop for extension workers and representatives of farmer groups in the worst hit district of Amolatar in 2011. From Amolatar, the training workshops were rolled over to other affected districts in Lango and Acholi sub regions of Uganda (Figure 5). A total of 40 sub counties from 8 districts benefited from the training. Seventy one participants were targeted per Sub County (total of 355 for each district). Among the seventy one trainees, the criteria required representation of at least extension, farmer groups and political leadership. After the training, those trained at level 1 organized and conducted further training (level 2) to increase the number of people (trainers) available within each sub county, who then took the training further down to the community/farmer level (level 3).

Training at level 2 and 3 were based on needs and resources available in each sub county. Training modules involved lectures and visits to selected field locations for practical demonstrations and it ensured that participants appreciated both foliar and root

tuber symptoms of CBSD on cassava varieties widely adopted in the zone. Additional training materials were provided during the training sessions. These included printed handouts and CBSD fact sheets. Progress in implementation of the training program was monitored regularly and adjustments were done as necessary. Follow up visits were made to sub counties to document level of adoption of available management options including CBSD tolerant variety MM 96/4271.

RESULTS

Progress achieved

At the time of planning the zonal training program (2011) CBSD had already been confirmed to be present in all the 15 districts of the northern agro-ecological zone of Uganda. None of these districts had the necessary manpower to support rapid response to the disease. A critical gap was noted especially regarding the involvement of extension and policy makers. By careful selection of participants for the CBSD training workshop, the CBSD initiative managed to increase the number of trained manpower in each sub county and importantly, also expanded the diversity of trained stakeholders to include extension leaders and policy makers. Farmer group representatives selected from parish level conducted further training for their group members thereby significantly increasing the number of knowledgeable stakeholders across the zone (Table 1), a more than 10 fold increase as compared to the number trained at sub county level (level 1). At the community level, where more emphasis is needed for effective CBSD management, over 3,000 stakeholders were sensitized on CBSD across the zone within one year. Considering that an estimated 2.7 people are affected or threatened by the CBSD pandemic within the 40 target sub counties, the over 3,000 stakeholders trained at grass root level was considerable. Although this ratio did not match the required critical mass for effective disease management, it was expected that gains on containing disease spread would be achieved if the knowledge acquired was put to use. There were, however, concerns on whether those trained would effectively

Table 1. Number of extension, policy makers and farmer group members (level 1 and 2) trained on CBSD management in nine sub-counties in northern Uganda.

District	Extension	Policy makers	Farmer group representatives
Lango sub region			
Amolatar	15	3	235
Alebtong	15	3	355
Dokolo	15	3	325
Apac	15	3	325
Oyam	15	3	325
Acholi sub region			
Pader	15	4	325
Agago	15	3	325
Gulu	15	3	325

apply the knowledge gained.

As a result of the training, 60% of the sub counties in Lango and 40% in Acholi sub regions who benefited from the training adopted CBSD tolerant variety MM96/4271 as a variety of choice for their farmers under food security program. Foundation seeds of MM96/4271 were obtained from Ngetta Zonal Agricultural Research and Development Institute and multiplied in farmer's locations with a pathological focus. This helped to increase access to CBSD tolerant varieties by smallholder farmers in the northern agro-ecological zone of Uganda, hence the decline in the spread of CBSD through movement of diseased planting materials.

Some challenges and lessons learnt

Absentee trainers

In numerous instances training at level 2 (parish level) was affected by loss of training capacity since some of those trained at level 1 (sub county level) were not available to conduct training at level 2. Notably, most of those selected to participate at level 1 were farmer group leaders or policy management staff who were not available to participate fully in further training, though they contributed to CBSD management in other ways. Based on this experience, it would be advisable to consider the ability and availability of trainees to participate in further training.

Communication

Extension workers and farmer groups have different levels of education. This required particular attention during joint training sessions to ensure participants followed and understood proceedings fully. This was addressed by providing translation to local languages since the facilitations were in English. However, attention needed to be paid to ensure key message was not

distorted or lost during translation, especially where technical details are involved. During this training, it was observed that it is generally difficult to translate. Field demonstration trips were included to reinforce learning through coursework. At the field level, differences in education levels were also noted to hinder direct interaction between workshop participants and farmers/other stakeholders who wanted to share their experiences on CBSD management directly. This was eased by having a translation service during field visits, though this may also not fully address the issue. As an alternative, it was suggested to consider holding separate workshops and training sessions for the extension and farmer group representatives.

Insufficient local capacity

The cascade training proposed assumed that each sub county had a basic functional extension system that would be trained and facilitated to further train farmers. The reality, however, was observed to be that there are considerable differences in capacities and institutional structures between sub counties, which affected program implementation. Whereas functional systems exist in Lango sub region, they are considerably weak and are almost non-existent in Acholi sub region. The Acholi Sub counties were especially facing challenges that were associated with civil instabilities experienced in the last two decades. These differences between capabilities of sub counties need to be taken into consideration when designing such training programs, and where possible support should be skewed to favor those in greater need.

Perception of CBSD as a non-threat

In Uganda, farmers react faster to threats that are perceived as current and immediate. The CBSD training program was designed to reach communities and stakeholders that were already affected and those threatened

but not yet affected by CBSD. With no previous experience of the devastating impact of CBSD, the threatened communities were unlikely to invest much effort to combat CBSD, even though they might have appreciated the lessons imparted through training. This slow response is particularly higher where cassava is not the primary means of supporting livelihoods. Exchange visits between farmers in different regions of Uganda could help in deepening appreciation of the threat and potentially encourage implementation of CBSD management measures.

DISCUSSION

Capacity building through the transfer of knowledge on CBSD symptom identification and management to stakeholders helped to build a coalition of local teams to combat CBSD in the zone. CBSD awareness campaign plays a vital role in checking the spread of the disease and has contributed to reviving cassava production in areas affected by the epidemic. At the time of initiating the awareness campaign, CBSD was already established in most parts of the zone. In addition to building capacity to recognize CBSD at sub county, parish and village levels, the training encouraged and promoted the use of available CBSD control programs, including the adoption of promising CBSD-tolerant cultivars particularly MM96 / 4271, production and distribution of clean cassava planting material. The two approaches are now widely adopted in the zone as a result of the awareness creation campaign. A number of multiplication sites for CBSD tolerant variety, MM96/4271 have been established within the zone to ensure control of movement of diseased cassava planting materials. In addition, all the sub counties were awareness creation were conducted; bylaws have been put in place to prevent distribution of diseased cassava cuttings to farmers. All cassava gardens intended for distribution to farmers are now first inspected by trained pathologists to assess their CBSD status. This is helping to curb CBSD spread through distribution of cuttings to farmers. However, complete recovery and the prevention of any further spread of CBSD are still a long way off. They require a strong commitment from local, national and international communities to sustain the ongoing and emerging research and development efforts that are devising effective and eco-friendly technologies for Uganda.

Conclusion

The implemented training program significantly increased the capacity of stakeholders across the zone to manage CBSD. The training strategy employed enhanced interaction between stakeholders within and between sub countries in the northern agro-ecological zone of Uganda. This has contributed to better coordination and management of CBSD through sharing of resources and experiences.

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Full Length Research Paper

Pollen morphology of seven wild species of *Acacia* in Saudi Arabia

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Pollen micromorphological characters such as pollen size, shape, number of associated monads, colpi and ornamentation of the tectum surface, of seven species of *Acacia* belonging to the family Mimosaceae were studied, using light microscope (LM) and scanning electron microscope (SEM). The study showed that the pollen size range from 42.1 to 69.4 μm , pollen shape is round to semi-round, the number of associated monads is 16 or 32, and the colpus is Y - H shaped. The tectum surface ornamentation among the species is variable, it is foveolate in *Acacia ehrenbergiana*; psilate-foveolate in *Acacia nilotica*, *Acacia laeta* and *Acacia negrii*; and micro-reticulate in *Acacia farnesiana*, *Acacia oerfota* and *Acacia tortilis* ssp. *raddiana*. An artificial key based on micromorphological characters is provided.

Key words: Mimosaceae, *Acacia*, pollen morphological characters, Saudi Arabia.

INTRODUCTION

Acacia Mill. belongs to the family Mimosaceae. It is widely distributed in tropical and subtropical regions (Elias, 1981). The subfamily Mimosoideae includes three tribes Acacieae Benth., Ingeae Benth. and Mimoseae Born. (Bentham, 1842). Tribe Acacieae includes only a single genus *Acacia* Mill. as stated by Bentham (1875). The number of species recorded in this genus is about 1400 species widely distributed in tropical and subtropical regions. Hopper and Maslin (1978) recorded 600 to 900 species in Western Australia while Chaudhary and Al-Jawaid (1999) suggested 1100 species in the world. However, only 12 to 16 species have been reported in the Kingdom of Saudi Arabia (Chaudhary, 2000; Collenette, 1999; Migahid, 1996).

Acacia Mill. is an economically important genus, all parts of various *Acacia* species are used for one purpose or another as sources of food, fodder, fire-wood and a variety of natural products, such as wood, gum exudates, tannins and honey (Chaudhary, 1983; Springuel and

Mekki, 1993; Al-Zoghet and Tag El-Din, 1995). Most of the *Acacia* species are of medicinal benefits to man and his livestock, e.g. *Acacia nilotica* produces arabic gum which is used for treating kidney diseases, and its pods are used for treating wounds and diarrhea (Elkhalifa, 1996).

Acacia pollen grains morphology has been studied by several investigators. Boulos L, 1983; Elias (1981) mentioned that the pollen characteristic features in Mimosoideae genera shows that they shed an individual persistent unit, as tetrad, octad, or polyad units, mostly of 16 and 32 monads. Guinet (1981) as recorded that the most structural pattern of the pollen grain is granular with common porate aperture, although, pollen grain with colporate and extraporate apertures are present, but the colpate aperture does not exist in pollen grains of the Mimosoideae. Jumah (1991, 1996) has reported spherical polyads of 16 grains in *Acacia karroo*; *A. nilotica* var. *adansoni*, *A. nilotica* var. *tomentosa* and *Acacia polyacantha* sub sp.

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Table 1. Sites, year of collection and pollen morphological characters of *Acacia* species under study. P = Polar axis (μm), E = equatorial diameter (μm), (P/E) = ratio of polar axis and equatorial diameter.

Specie	Pollen size LM (μm)		(P/E)	Pollen shape	Number of monad	Type of colpi	Tectum surface	Sites and year collection
	P	E						
<i>A. ehrenbergiana</i>	49.8	49.2	1.01	Rounded	16	Colporate	Foveolate	Al-Hada, 2005
<i>A. farnesiana</i>	67.9	69.4	0.98	Semi-rounded	32	Colporate	Micro-reticulate	Al-Baha, 2001
<i>A. laeta</i>	42.1	44.1	0.96	Rounded	16	Porate	Psilate-foveolate	Al-Baha -Bin Saad rood, 2005
<i>A. negrii</i>	45.4	44.8	1.01	Semi-rounded	32	Colporate	Psilate-foveolate	Abha, 2003
<i>A. nilotica</i>	50	48	1.04	Rounded	32	Colporate	Psilate-foveolate	Abha, 2006
<i>A. oerfota</i>	64.8	62.4	1.04	Semi-rounded	32	Porate	Micro-reticulate	Abha, 2001
<i>A. tortilis</i> ssp. <i>raddiana</i>	57	56.4	1.01	Rounded	32	Colporate	Micro-reticulate	Beasha, 2003

campylacantha. Guinet (1990) noted that the pollen structural symmetry was shared by some Mimoseae and *Acacia*. Kordofani and Ingrouille (1992) studied 14 species of *Acacia* reporting that the pollen grains in each polyad are 16 except in one species which has 32 grains in each polyad. Fitzgerald et al. (1993) studied the development and initiation of cohesion between compound pollen grains of *Acacia paradoxa* and found special cases of pollen development. Robbertse (1974) examined the surface structures of pollen grains of *Acacia giraffae* and *Acacia faidherbia* in a study to clarify their taxonomic relationship. Perveen and Kaiser (1998) also studied the pollen grains of five species of *Acacia* in Pakistan and revealed that the pollen morphology differences of the subfamily Mimosoideae is significant at the generic and tribal level. Caccavari and Dome (2000) investigated morphological and structural characterization of pollen grains in 77 American species of *Acacia* and suggested that the pollen features can be used as a distinguishing factor of the generic restrictions for *Acacia*. Tantawy et al. (2005) also studied the pollen morphological characters in 14 species of *Acacia* in Egypt and reported that the morphological features of the pollen grains are indicative of generic and specific level of studied Mimosoideae. El Azab (2005) suggested that some *Acacia* species may be differentiated into different groups according to their pollen characters. Recently, Rajurkar et al., (2013) observed that *A. nilotica* and *Acacia leucophloea* polyads had 16 pollen grains and the pollen variations in their morphological characters for example, the shape, size, surface pattern and surface structure which was found to be significantly helpful at generic or specific level. The pollen of *Acacia* species in Saudi Arabia has limited investigations regarding the honey and allergy. So the aim of this study was to investigate in details the micro morphological characters of pollen grains of some *Acacia* species to show how far the pollen morphological

variations could be used to distinct between the studied species of *Acacia* which are growing naturally in Saudi Arabia.

MATERIALS AND METHODS

The pollen grains of 6 species and one subsp. of *Acacia* growing wildly in Saudi Arabia were obtained from specimens in the Herbarium of the Ministry of Agriculture and Water, Riyadh, Saudi Arabia (Table 1 and Figure 22). Pollen grains for light microscope (LM) examination were prepared using the usual acetolysis method (Erdtman, 1960; Moore et al., 1991) and mounted in either glycerin gelatine or glycerin. Observations were made using Olympus CH20 Microscope, and photographs were taken using Olympus BX41TF with camera video TK-C1381EG. For scanning electron microscope (SEM) studies, pollen grains samples were run through an alcohol series: 50, 70, 80, 95, 100 % then mounted on the stubs using micro-pipettes. The stubs were coated usually to 30 nm with gold and then the pollen grains were ready for scanning (Punt, 1962). The representative pollen grains were photographed at various magnifications in a JSM-5800 LV (JOEL) scanning electron microscope. The measurements were based on 20 reading from each specimen. Descriptive terms were according to Moore and Webb (1978) and measurements for the pollen grains size of studied species were taken according to Erdtman (1960) [very small < 10 μm in polyad diameter; small 10-25 μm ; medium (25-50) μm ; large (50-100) μm ; very large (100-200)].

RESULTS AND DISCUSSION

Representative pollen grains of 7 *Acacia* species are illustrated in Figures 1 to 21. Pollen morphological characters and measurements are provided in Table 1. The pollen shape is round in *Acacia ehrenbergiana*, *Acacia laeta*, *A. nilotica* and *Acacia tortilis* ssp. *raddiana* (Figures 1, 3, 5, 7; Table 1), this result confirms that mentioned by Kordofani and Ingrouille (1992), Tantawy et al. (2005) and Rajurkar et al. (2013). Shape is semi-round in *Acacia farnesiana*, *A. negrii* and *A. oerfota*

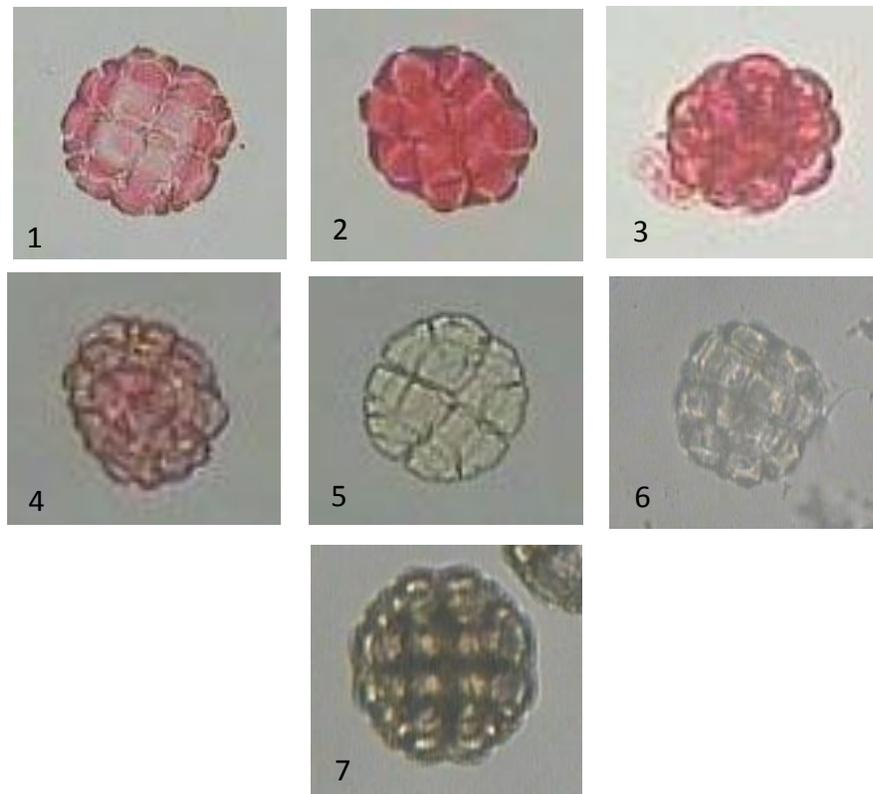


Figure 1-7. LM micrographs showing pollen shapes. 1: *Acacia ehrenbergiana* pollen round; 2: *A. farnesiana*, pollen semi-round; 3: *A. laeta*, pollen round; 4: *A. negrii*; pollen semi-round; 5: *A. nilotica*, pollen round; 6: *A. oerfota*, pollen semi-round; 7: *A. tortilis* ssp. *raddiana*, pollen round. 400x.

(Figures 2, 4, 6; Table 1), and this in an agreement with report of Tantawy et al. (2005). The pollen size is large in *A. farnesiana*, *A. oerfota* and *A. tortilis* ssp. *raddiana*; and medium in *A. ehrenbergiana*, *A. laeta*, *A. negrii* and *A. nilotica* (Table 1). This result is almost similar to that reported by Tantawy et al. (2005) and Kordofani and Ingrouille (1992) on the pollen grains of *A. tortilis* ssp. *raddiana* and almost similar to that reported by Rajurkar et al. (2013) on the pollen grains of *A. nilotica*. The number of associated monads are 16-monads in *A. ehrenbergiana* and *A. laeta* (Figures 8 and 12, respectively), and 32-monads in *A. farnesiana*, *A. negrii*, *A. nilotica*, *A. oerfota*, and *A. tortilis* ssp. *raddiana* (Figures 10, 11, 14, 16, 18, 20 respectively and Table 1). This result is similar to that mentioned by Caccavari and Dome (2000) in their key of the pollen types and subtypes of American *Acacia*, and Rajurkar et al. (2013) on the pollen grains of *A. nilotica*. But it conflicts with that reported by Tantawy et al. (2005) since in the latter studies, all species have pollen grains with 32 monads (Figures 2, 4-7 and 10, 11, 14-21) except *A. ehrenbergiana* and *A. laeta* which have polyads (pollen grains) with 16 monads (Figures 1, 3, 8, 9, 12 and 13). Our result also disagree with that reported by Kordofani and Ingrouille (1992) about the presence of 16 monads in each polyad of *A. nilotica* and *A. tortilis* ssp. *raddiana*

(Figures 5, 16, 17 and 7, 20, 21, respectively) but agree with their finding on each polyad of *A. ehrenbergiana* with 16 monads (Figures 1, 8 and 9). The conflict in the number of monads in each pollen of *A. nilotica* and *A. tortilis* ssp. *raddiana* which grow in South West of Saudi Arabia and those grown in Sudan and Egypt may be due to the differences in environmental conditions in each country. The species of *Acacia* are characterized by pollen grains with colpi of Y and H-shape, that is, grooves-like at central and peripheral of the pollen grains provided with pores, this distinguishing character is used to identify the American *Acacia* species (Caccavari and Dome, (2000). However, the type of colpi is colporate in *A. ehrenbergiana*, *A. farnesiana*, *A. negrii*, *A. nilotica*, *A. tortilis* ssp. *raddiana* (Figures 9, 11, 15, 16, 19, 21 respectively and Table 1) and porate in *A. laeta* and *A. oerfota* (Figures 13, 19 and Table 1) this observation is in an agreement with that reported by Guinet (1981b). The tectum surface is foveolate in *A. ehrenbergiana* (Figure 9 and Table 1); psilate in *A. laeta* and *A. negrii* (Figures 13, 15 and Table 1); psilate-foveolate in *A. nilotica* (Figure 16 and Table 1); and micro-reticulate in *A. farnesiana*, *A. oerfota* and *A. tortilis* ssp. *raddiana* (Figures 11, 19, 21 and Table 1). This result is similar to the observations of pollen of 16 or 32 monads confirmed that of Elias (1981)

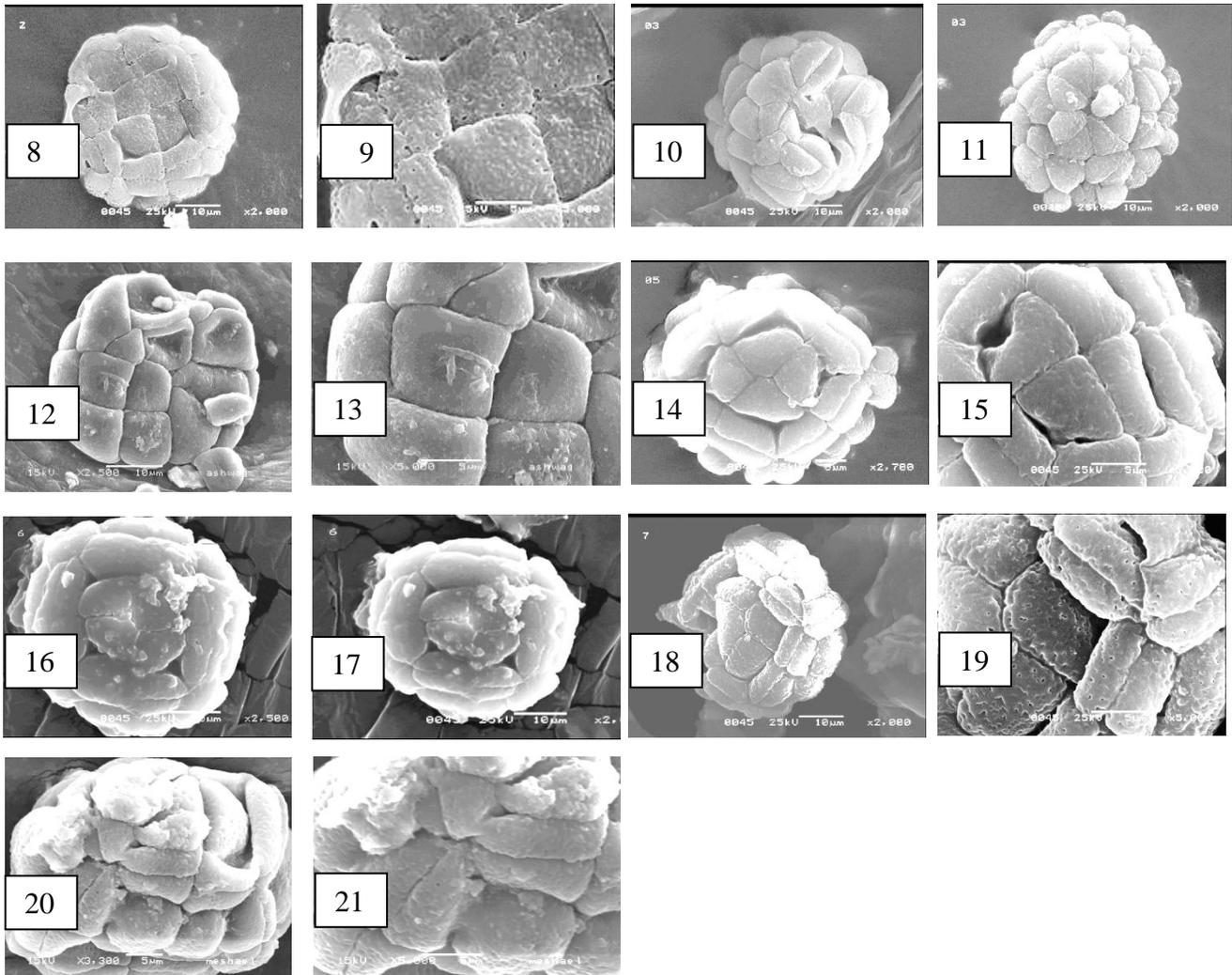


Figure 8-21. SEM micrographs showing pollen structure. 8, 9: *Acacia ehrenbergiana*; pollen in polar view. 10, 11: *A. farnesiana*, pollen in polar view. 12, 13: *A. laeta* pollen in polar view. 14, 15: *A. negri* pollen in polar view. 16, 17: *A. nilotica* pollen in polar view. 18, 19: *A. oerfota* pollen in equatorial view. 20, 21: *A. Tortilis* ssp. *raddiana*, pollen in polar view.

who indicated in his study that the pollen in Mimosoideae genera, shed single units of persistent tetrad, octad, or polyad units, mainly of 16 or 32 grains. We can divide the seven investigated species into two groups based on type of colpi and the number of polyads cells. The first group is polyad of 16 monads including *A. ehrenbergiana* and *A. laeta* which can be distinguished by the type of colpi, which is colporate in the pollen grains of *A. ehrenbergiana* (Figure 9 and Table 1) and porate in the pollen grains of *A. laeta* (Figure 13 and Table 1). The second group, polyad of 32 monads includes the other 5 species viz. *A. oerfota*, *A. negrii*, *A. farnesiana*, *A. nilotica* and *A. tortilis* ssp. *raddiana*. However, *A. oerfota* can be separated by its pollen grains with porate type (Figure 19 and Table 1) and the four remaining species are with colporate pollen grains (Figures 11, 13, 15, 21 and Table 1). Tectum surface of the pollen grains can also be used

to divide the last four species into two groups, pollen grains with micro-reticulate surface includes *A. farnesiana* and *A. tortilis* ssp. *raddiana* (Figures 13, 21, respectively and Table 1) and pollen grains with psilate-foveolate surface includes *A. negri* and *A. nilotica* (Figures 15, 16, respectively and Table 1). Pollen grains size shows a great variation between the last four species, where *A. farnesiana* pollen size is 67.9-69.4 μm and *A. tortilis* ssp. *raddiana* pollen size is 57-56.4 μm , while *A. negrii* pollen size is 44.8- 45.4 μm and *A. nilotica* pollen size is 48-50 μm , which can be used to distinguish between them (Table 1).

Key based on the morphological characters of pollen grains of investigated *Acacia* species which grow naturally in Saudi Arabia as proposed:

I- Polyad, 16 monads



Figure 22. Collection sites of *Acacia* species in Saudi Arabia.

- 1-Type of colpi, Colporate *A. ehrenbergiana*
- 2- Type of colpi, Porate *A. laeta*

II- Polyad, 32 monads

- 1- Type of colpi, Porate *A. oerfota*
- 2-Type of colpi, Colporate

A- Tectum surface, micro-reticulate

- 1- Pollen size (57-56.4 μm) *A. tortilis* ssp. *raddiana*
- 2 - Pollen size (67.9-69.4 μm) *A. farnesiana*

B- Tectum surface, psilate-foveolate

- 1- Pollen size (48-50 μm) *A. nilotica*
- 2- Pollen size (44.8- 45.4 μm) *A. negrii*

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Full Length Research Paper

Petiole anatomy of some species of Asteraceae in southwest Nigeria

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Petiole anatomy of 12 species of Asteraceae around Ile-Ife in South-Western Nigeria was described. Transverse section of the petiole (median) was cut at 20 μ using Reichert Sledge Microtome. The specimens were stained in 1% aqueous solution of Safranin O for 5 min, washed in three changes of water to remove excess stain and counterstained in 1% solution of Alcian blue for 5 min, and cleared in xylene. The section was mounted in DPX. The distinguishing characteristics of taxonomic value include, shapes of the petioles, variation in the number, arrangement and shapes of vascular bundles, types of trichomes.

Key words: Asteraceae, petiole, anatomy, taxonomic, trichomes.

INTRODUCTION

Asteraceae is a very large cosmopolitan family, highly advanced and easily recognized with worldwide distribution (Nielsen, 1965). It belongs to the sub-class Asteridae in the order Asterales (Ming, 1999). Asteraceae is the second largest family in the division Magnoliophyta with 1,100 genera and over 20,000 recognized species (Ming, 1999). The majority of Asteraceae species are herbaceous although an important component of the family consists of shrubs or even trees, many plants in the family Asteraceae are economically important as weeds, ornamentals, medicinal and green vegetables (Olorode, 1984).

Angiosperms are endowed with external morphological characters of significant taxonomic value which can be easily observed with the naked eye or with simple hand lens. Morphological attributes of vegetative organs have often constituted the mainstay of taxonomic studies in plants (Polhill, 1968; Pilbeam and Bell, 1979; Adedeji, 2005) and are very important in classification.

The use of anatomical methods in taxonomic investigations cannot be over emphasized. Although no character is absolutely immutable, some are more fixed than the others and it is on those that are less plastic that

the systematic anatomist rely because they are not really affected by environmental conditions (Barthlott, 1981); comparative plant epidermal studies have been found to be reliable in taxonomy and systematics (Stace, 1969; Ogunkunle and Oladele, 2000). Metcalfe and Chalk (1950, 1979), Naik and Nigrude (1981), Palmer and Tucker (1981), Adedeji (2004) and Adedeji and Illoh (2004) have all stressed the taxonomic importance of anatomical features which along with other characters are useful for identification and classification of plants.

The present study reports on the use of petiole anatomy in establishing the taxonomic relationships among ten species of Asteraceae.

MATERIALS AND METHODS

Transverse section (median) of the petiole of *Ageratum conyzoides* Linn., *Aspilia africana* (Pers) C.D Adams, *Bidens pilosa* Linn., *Chromolaena odorata* (Linn.) King & Robinson, *Crassocephalum crepidioides* Benth S. Moore, *Synedrella nodiflora* (Linn) Gaertn, *Tithonia diversifolia* (Hemsl) A. Gray, *Tridax procumbens* Linn. *Vernonia amygdalina* Del., *Vernonia cinerea* Linn were cut at 20 μ using Reichert sliding microtome. The sections were preserved

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in 50% ethanol. The sections were stained in 1% aqueous solution of Safranin O for 5 min, washed in three changes of water to remove excess stain and counterstained in 1% solution of Alcian blue for 5 min. The sections were then washed in three changes of water and dehydrated by passing them through series of ethyl alcohol: 50, 70, 80, 90 and 100% with two changes in 100% alcohol to remove water molecules, (dehydration process) and excess stain (differentiation process). The dehydrated and differentiated sections were cleared in xylene and mounted in DPX. Photomicrographs of the petiole sections of the ten species were taken with Amscope digital camera attached to a light microscope. All microscopic measurements were made and expressed in micrometer.

RESULTS

***Ageratum conyzoides* Linn. (Plate 1A)**

Outline: Arc shape, adaxial side concave, abaxial surface convex. Epidermis: Uniseriate. Cortex: Compose of 1-2 layers of polygonal angular collenchyma cells and 11-14 layers of polygonal parenchyma cells. Vasculature: Vascular bundles, 3-5, bicollateral, arranged in form of an arc. Pith: Consists of thin walled parenchyma cells. Trichome type: Simple, uniseriate and multicellular.

***Aspilia africana* Pers. C.D. Adams (Plate 1B)**

Outline: Arc shape, concave on the adaxial surface, convex in the abaxial. Epidermis: Uniseriate. Cortex: Compose of 4 layers of polygonal angular collenchyma cells and 5 layers of thin-walled spherical shaped parenchyma cells. Vasculature: Vascular bundles, 4, consisting of 3 prominent bicollateral bundles alternating with a small one. Rib bundle is also present. Pith: Consist of thin walled parenchyma cells. Trichome types: Simple, uniseriate and multicellular.

***Bidens pilosa* Linn. (Plate 1C)**

Outline: Concave at the adaxial surface, convex at the abaxial. Epidermis: Uniseriate. Cortex: Compose of 2-3 layers of polygonal angular collenchyma cells and 5 layers of thin walled parenchyma cells. Vasculature: Vascular bundles, 2-4, amphicribal arranged in form of an arc. Pith: Thin walled parenchyma cells present. No trichome present.

***Chromolaena odorata* Linn. (Plate 1D)**

Outline: Boat shaped, slightly concave at the adaxial surface, convex on the abaxial surface. Epidermis: Uniseriate. Cortex: Compose of 2-3 layers of polygonal angular collenchyma cells and 4 layers of thin walled parenchyma cells. Vasculature: Vascular bundle, 3, arranged in form of an arc, amphicribal. Pith: Thin walled

parenchyma cells present. Trichome types: Simple, uniseriate multicellular.

***Crassocephalum crepidioides* (Benth) S. Moore. (Plate 1E)**

Outline: Concave on the adaxial surface, convex on the abaxial surface. Epidermis: Uniseriate. Cortex: Compose of 2-3 layers of polygonal angular collenchyma cells and 6-7 layers of thin walled parenchyma cells. Vasculature: Vascular bundles 3-5, amphicribal arranged in the form of an arc. Pith: Consist of thin walled parenchyma cells. No trichome present.

***Synedrella nodiflora* Benth. (Plate 2A)**

Outline: Arc shape, concave at the adaxial surface, abaxial surface is convex. Epidermis: Uniseriate. Cortex: Compose of 2-4 layers of polygonal angular collenchyma cells and 5-6 layers of parenchyma cells. Vasculature: Vascular bundles, 5-7, bicollateral. Pith: Consist of thin walled parenchyma cells. No trichome present.

***Tithonia diversifolia* Hemsl. A Gray (Plate 2B)**

Outline: Concave at the adaxial surface, abaxial surface is convex. Epidermis: Uniseriate. Cortex: Compose of 3 layers of polygonal angular collenchyma cells and 6 layers of thin walled parenchyma cells. Vasculature: Vascular bundles, 3-5, bicollateral arranged in the form of an arc. Pith: Thin walled parenchyma cells present. Trichome types: Simple, uniseriate and multicellular.

***Tridax procumbens* Linn. (Plate 2C)**

Outline: Boat shape, concave at the adaxial surface, abaxial surface is convex. Epidermis: Uniseriate. Cortex: Compose of 2-3 layers of polygonal angular collenchyma cells and 4-5 layers of thin walled parenchyma cells. Vasculature: Vascular bundles, 3, amphicribal arranged in form of an arc. Pith: Thin walled parenchyma cells present. Trichome types: Simple, uniseriate and multicellular.

***Vernonia amygdalina* Del. Cent. (Plate 2D)**

Outline: Slightly concave at the adaxial surface, abaxial surface is convex. Epidermis: Uniseriate. Cortex: Compose of 2-3 layers of polygonal angular collenchyma cells and 6-7 layers of polygonal parenchyma cells. Vasculature: Vascular bundles, 2-3, bicollateral. Pith: Thin walled parenchyma cells present. No trichome

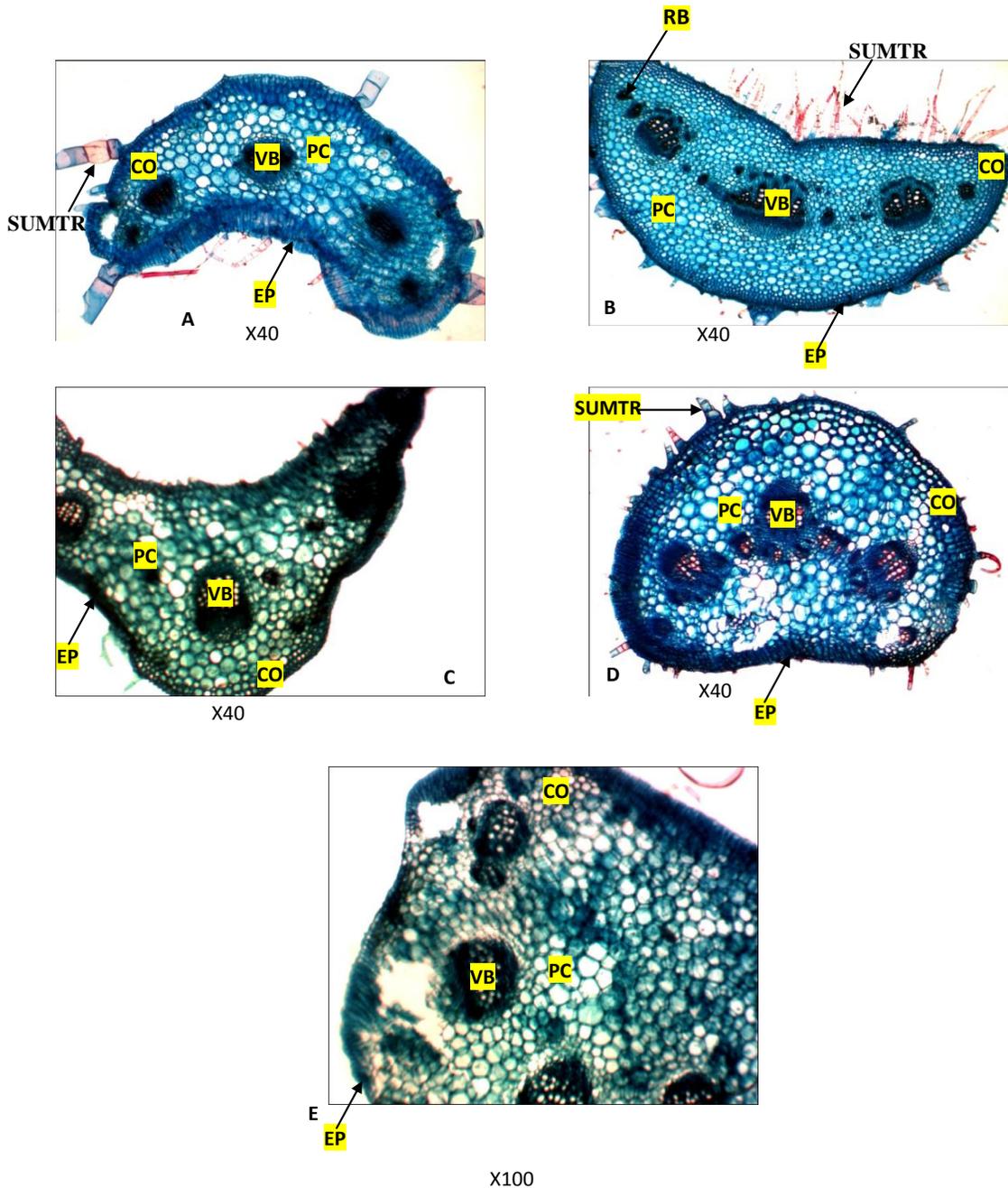


Plate 1. A- *Ageratum conyzoides*; B- *Aspilia africana*; C- *Bidens pilosa*; D- *Chromolaena odorata*; E- *Crassocephalum crepidioides*. BTR- Bicellular trichome; EP- epidermis; CO- collenchyma; PC- parenchyma; RB- rib bundle; SUMTR- simple uniseriate multicellular trichome; VB- vascular bundle.

present.

***Vernonia cinerea* Linn. (Plate 2E)**

Outline: Slightly concave at the adaxial surface, abaxial surface is convex. Epidermis: Uniseriate. Cortex: Compose of 1-2 layers of polygonal angular collenchyma cells and 5-7 layers of polygonal parenchyma cells.

Vasculature: Vascular bundles, 3, oval shaped, amphicribal. Pith: Thin walled parenchyma cells present.

DISCUSSION

The data recorded are useful to distinguish the taxa studied because each taxa showed unique anatomical characters which are diagnostic, for examples, the shape,

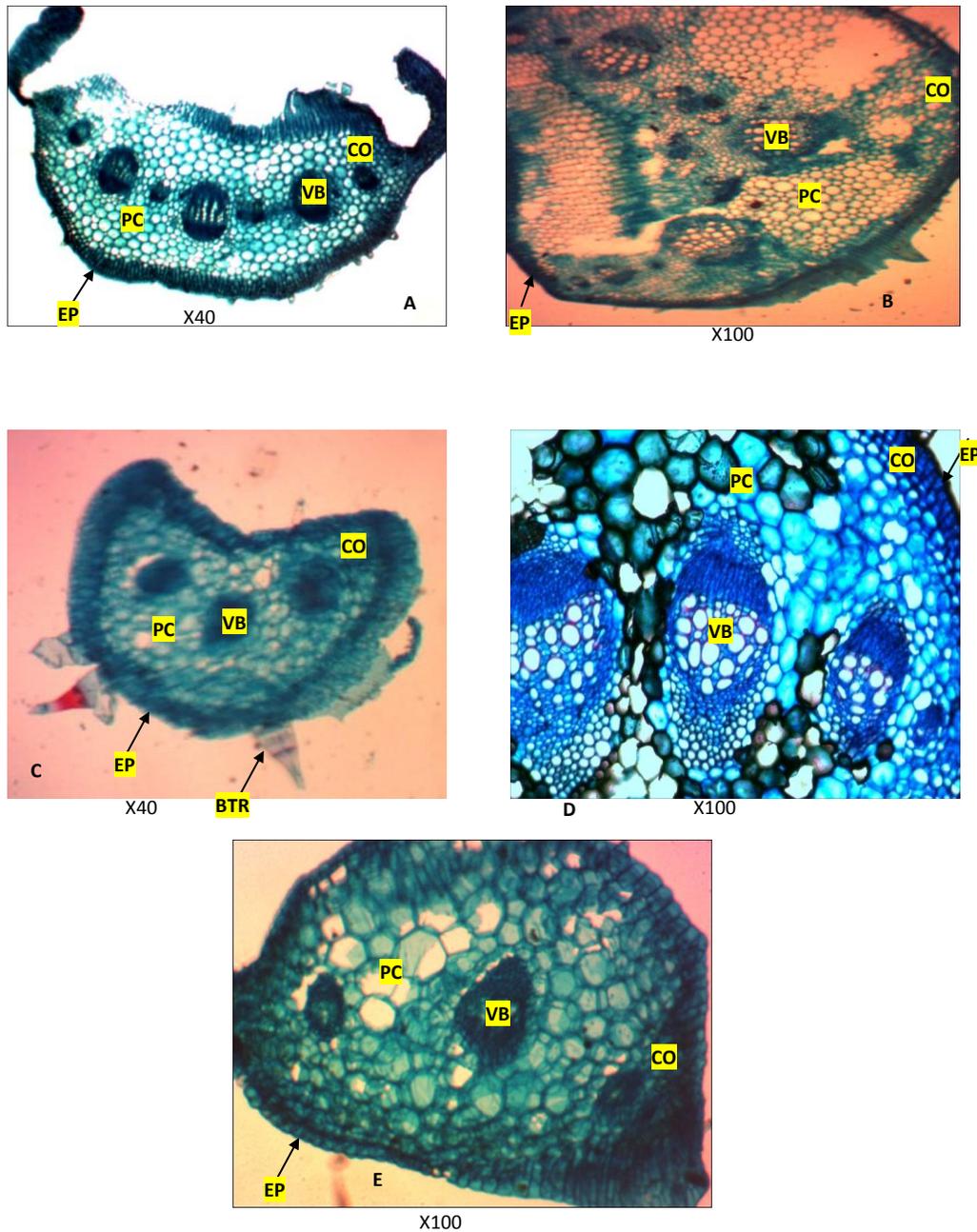


Plate 2. A- *Synedrella nodiflora*; B- *Tithonia diversifolia*; C- *Tridax procumbens*; D- *Vernonia amygdalina*; E- *Vernonia cinerea*. BTR- Bicellular trichome; EP- epidermis; CO- collenchyma; PC- parenchyma; RB- rib bundle; SUMTR- simple uniseriate multicellular trichome; VB- vascular bundle.

layers of their collenchyma and parenchyma cells, arrangements of vascular bundles and trichome distributions, even though overlaps were observed in some of the species. Many researchers have utilized anatomy for taxonomic consideration in many species of plants. These include the works of Naik and Nigrude (1981) on *Chlorophytum* Linn., Ogundipe and Olatunji (1991) on *Cochlospermum* Linn., Illoh (1995) on *Celosia* Linn., Adedeji (2004) on *Emilia* Cass. and Adedeji and Illoh (2004) on *Hibiscus* Lyndley Craven.

Anatomy of the transverse sections of the petiole of species studied reveals both intra and interspecific variations which are important in the classification and delimitation of the species. The pattern of distribution and composition of tissues are uniform in all the species studied with few exceptions, that is, the shape, layers of their collenchyma and parenchyma cells, arrangements of vascular bundles. However, the differences observed in the outline of the adaxial surface and the arrangement and types of vascular bundles can be used to separate

the genera of the family Asteraceae studied. Generally, the epidermis is adaxially concave, uniseriate and made up of angular collenchyma cells while polygonal cells are found in the cortex. These appear to be characteristic of the family. Anatomical distinctions occur in the vascular bundles of the species studied. The distinction in shape and type of vascular bundle in the species studied are of taxonomic value because it divided the species studied into two different groups, that is, amphicribal and bicollateral with differences in the number of bundles. Vascular bundles are either joined together or broken into two and are arranged in the form of an arc. Two major types of vascular bundles occur in the species studied: amphicribal and bicollateral bundles. These are useful in dividing the species studied into two groups. Amphicribal bundles are found in *B. pilosa*, *C. odorata*, *Crassocephalum crepidioides*, *S. nodiflora*, *T. procumbens* and *V. cinerea* while bicollateral bundles are found in *A. conyzoides*, *A. africana*, *T. diversifolia* and *V. amygdalina*. *A. conyzoides* and *T. diversifolia* are different from the other species studied, the vascular bundles are not joined (they are referred to as open); they possess 3-5 vascular bundles. The vascular bundles of *A. africana* with three prominent large vascular bundles alternate with small ones and possess rib bundles. These separate *A. africana* from other species. *S. nodiflora* is quite distinct from others because of numerous bundles.

The presence or absence of trichomes in the epidermis of the petioles of the species studied is taxonomically valuable. Based on the presence or absence of trichomes, the species studied can be grouped into two. Trichomes are found in *A. africana*, *A. conyzoides*, *C. odorata*, *T. diversifolia* and *T. procumbens*. Trichomes are absent in *B. pilosa*, *C. crepidioides*, *S. nodiflora*, *V. amygdalina* and *V. cinerea*. In species where trichomes are found they are simple uniseriate and multicellular.

Artificial key to the species of Asteraceae using anatomical characters

- 1a. Trichomes present, simple multicellular non glandular
 2a. Rib bundle present.....
A. africana
 2b. Rib bundle absent
 3a. Petiole outline boat shaped..... *C. odorata*
 3b. Petiole outline arc shaped
 4a. Cortex made up of 11-14 layers of polygonal parenchyma cells. *A. conyzoides*
 4b. Cortex made up of 4-6 layers of parenchyma cells
 5a. Vascular bundle amphicribal.....
T. procumbens
 5b. Vascular bundle bicollateral..... *T. diversifolia*
 1b. Trichomes absent

- 6a. Vascular bundle bicollateral
 7a. Outline slightly concave on adaxial..... *V. amygdalina*
 7b. Outline arc shaped on the adaxial surface..... *S. nodiflora*
 6b. Vascular bundle amphicribal
 8a. Vascular bundles 3.....
V. cinerea
 8b. Vascular bundles 2-5
 9a. 5 layers of parenchyma cells present in the pith..... *B. pilosa*
 9b. 6-7 layers of parenchyma cells on the pith..... *C. crepidioides*

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Full Length Research Paper

Effect of intra-row spacing on yield and quality of some onion varieties (*Allium cepa* L.) at Aksum, Northern Ethiopia

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Lack of improved varieties and production practices have been the major bottlenecks of onion production and productivity in Tigray, particularly at Aksum area. There have been no recommended intra-row spacing and variety for that area specifically; rather farmers used to practice non-uniform plant spacing. Thus, a field experiment was conducted to investigate the influence of intra-row spacing, variety and their interactions on yield, shelf life and bulb quality of onion, thereby recommend the optimum practices to farmers in the study area. The study was conducted between August 2010 and April 2011 at Aksum area (L/maichew district). Three different intra-row spacings (5, 7.5 and 10 cm) were evaluated using four varieties of onion ('Adama' Red, 'Bombay' Red, 'Melkam' and 'Nasik' Red) using RCBD replicated four times. Data on yield and quality parameters were recorded and subjected to ANOVA. Results indicate that intra-row spacing of 10 cm was superior in plant height, leaf number per plant, leaf biomass yield, leaf dry matter content and percentage of bolters. Highest total bulb yield was recorded at the closest intra-row spacing (5 cm) followed by 7.5 cm. 'Melkam' variety was the highest yielder, while 'Adama' Red was the lowest yielder. Average bulb weight increased with increasing intra row spacing. 'Melkam' variety followed by 'Bombay' Red variety was superior in average bulb weight. 'Adama' Red recorded the highest unmarketable yield.

Key words: Intra-row spacing, yield, quality, onion varieties, spacing.

INTRODUCTION

Onion (*Allium cepa* L.) belongs to the genus *Allium* of the family Alliaceae (Hanelt, 1990). Onion is by far the most important of the bulb crops cultivated commercially in most parts of the world. The crop is grown for consumption both in the green state as well as in mature bulbs. Onions exhibit particular diversity in the eastern Mediterranean countries, through Turkmenistan, Tajikistan to Pakistan and India, which are the most important sources of genetic diversity and believed to be center of

origin (Astley et al., 1982; Brewster, 2008). *Alliums* are typically plants of open, sunny, dry sites in fairly arid climates, however many species are also found in the steppes, dry mountain slopes, rocky or stony open sites, or summer dry, open, scrubby vegetation (Hanelt, 1990).

Onion is considerably important in the daily Ethiopian diet, mostly used as seasonings or as vegetables in stews (MoARD, 2009). It is one of the richest sources of flavonoids in the human diet and flavonoid consumption

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has been associated with a reduced risk of cancer, heart disease and diabetes. In addition it is known for anti bacterial, antiviral, anti-allergenic and anti-inflammatory potential. One onion quality parameter, the percentage of single-center bulbs, has become important to meet demands of both processing and fresh market buyers (Brewster and Rabinowitch, 1990).

Yield and quality of dry bulbs can be influenced by cultural practices and growing environments. So far, research in the country was mainly focused on the identification of superior cultivars of onions and adopting improved management practices for better yield. The control of plant spacing is one of the cultural practices to control bulb size, shape and yield (Geremew et al., 2010).

The higher yield and better control of over or under bulb size could be obtained if plants are grown at optimum density. Bulb neck diameter, mean bulb weight and plant height decreased as population density increased.

Total bulb yield can be increased as population density increases (Kantona et al., 2003). Aksum-Adwa area is one of the potential areas for onion production in Ethiopia (EHDA, 2011). Market problem and poor cropping pattern are major problems in the study areas due to lack of proper agronomic practice used by farmers (AxARC, 2009). This is because there had been no agronomic or varietal trial done for onion so far at the area especially oriented to quality bulb production.

According to Fekadu and Dandena (2006), one of the major constraints of the vegetable sector in the country include: Lack of proper post harvest handling, suitable marketing and transportation systems, sufficient quantity of seed supply and good orientation of people to make them aware of the nutritive and economic advantages of these crops.

The present study was therefore undertaken to investigate the effects of different intra-row spacing on the yield and quality of onion varieties.

MATERIALS AND METHODS

Description of the study site

The study was conducted in 2010/11 from August 2010 to April 2011 at Aksum area (L/maichew district), Central Zone of Tigray National Regional State, 245 km away from Mekelle towards the North West. The experimental site lies between latitude of 14° 07' 00" and 14° 09' 20" N, and 38° 38' 00" and 38° 49' 09" E longitude, and elevation of 2080 m above sea level. The soil is classified as loamy clay vertisol. The rainy season of the area is monomial and receives 700 mm average rainfall per annum. The annual minimum and maximum monthly temperature ranges from 11 to 15.1°C respectively.

Experimental treatment and design

The experiment consisted of factorial combination of two factors of:

intra-row spacing (5, 7.5 and 10 cm) having plant population of 100, 75 and 50 per m² respectively and variety (Adama Red, Bombay Red, Melkam and Nasik Red). The row spacing was 20 cm. The field experiment was laid out in 3 x 4 factorial randomized complete block design (RCBD) replicated four times.

Experimental management

Cultural management practices other than intra-row spacing were done according to the national recommendations. During maturity when 2/3 of the leaves become yellow in color, bulb was harvested and cured for 5 days (EIAR, 2007). Five sample bulbs were taken from each plot for data collection at one time.

Data collection and analysis

Data were collected using the standard procedures described by IPGRI (2001). Total bulb yield, unmarketable bulb yield (UMY), marketable bulb yield (MBY), size category of bulbs (%), average bulb weight (ABW), bulb length (BL), bulb diameter (BD), neck diameter (ND), bulb dry matter content and total soluble solids (%) were measured and the mean values subjected to the Analysis of Variance (ANOVA) using SAS version 9.2 Computer software (SAS Institute Inc., 2008). Whenever the treatment was significant, least significance differences (LSD) was used for mean separation at $p=0.05$.

RESULTS AND DISCUSSION

Total bulb yield (t/ha)

Results indicated that there was no significant interaction effect between the intra-row and variety, while main effects of intra-row spacing ($p<0.0001$) and varieties ($p<0.01$) significantly influenced total bulb yield of onion. As intra-row spacing increased from 5 to 10 cm, total bulb yield in tons/hectare decreased. Significantly, the highest total bulb yields of 36.14 and 33.82 t/ha were recorded at 5 and 7.5 cm intra-row spacing, respectively. An intra-row spacing of 10 cm showed the lowest total bulb yield (28.51 t/ha) (Table 1). This is due to the reality that as intra-row spacing decreases, total plant population increases and this in turn contributes to increase in total bulb yield, but the bulb dimension and weight decrease. The current result is in agreement with works of different authors. Jan et al. (2003) recorded the highest yield (40.44 t/ha) at spacing of 17 x 4.5 cm, and the lowest yield (19.95 t/ha) at 27 x 14.5 cm spacing. Hassan (1978), Mohamedali (1988) and Russo (2008) also found similar results. Rekowska and Skupien (2007) also reported significantly higher yield of bulbs and green leaves of garlic in closer intra-row spacing.

Moreover, Kantona et al. (2003) noticed that onion yield increased from 17.4 to 39.5 t/ha as plant population per square meter increased from 50 to 150. Carlson et al. (2009) reported influence of plant density on the yield of two potato varieties, in which both varieties produced highest total yields at the closest plant spacing of 17.75 cm. Hemphill (1987) also reported that a fourfold increase

Table 1. Effect of intra-row spacing and variety on total bulb yield (TBY), marketable bulb yield (MBY) and average bulb weight (ABW) of onion.

Treatment	TBY (T/ha)	MBY (T/ha)	ABW(g)
Intra-row spacing (cm)			
5	36.14 ^a	34.49 ^a	49.86 ^c
7.5	33.82 ^a	32.97 ^a	69.69 ^b
10	28.51 ^b	28.10 ^b	81.31 ^a
LSD _(0.05)	3.10	3.10	4.76
CV (%)	12.90	13.63	9.90
Variety			
Adama Red	29.86 ^c	28.45 ^c	61.03 ^c
Bombay Red	34.68 ^{ab}	34.00 ^{ab}	67.29 ^b
Melkam	35.20 ^a	34.36 ^a	75.77 ^a
NasikRed	31.57 ^{bc}	30.60 ^{bc}	63.74 ^{bc}
LSD _(0.05)	3.53	3.60	5.50
CV (%)	12.90	13.63	9.90

Means within a column followed by the same letter(s) are not significantly different at $p \leq 5\%$ according to LSD test.

in planting density doubled the yield of shallot. The author further stated that yield per unit area did not increase proportionally to the increase in planting density since bulb weight per plant decreased at higher densities, but low planting density and small planting stock size favored production of large bulbs required for some markets, but with greatly reduced total yield.

Results also indicated that '*Melkam*' and '*Bombay*' Red varieties had the highest total bulb yield (35.20 and 34.68 t/ha), but '*Bombay*' Red fell into the second group with '*Nasik*' Red (31.57 t/ha), besides the second group '*Nasik*' Red also fell into the lowest group with '*Adama*' Red (Table 1). Significantly, the least total bulb yield (29.86 and 31.57 t/ha) was recorded for '*Adama*' Red but statistically similar with '*Nasik*' Red. The present finding is supported by different investigations previously done. Jilani and Ghaffoor (2003) and Jilani et al. (2009) suggested that varieties could have different yield potential in different agro-ecologies due to their genetic potential and genetic environment interaction effect.

Marketable bulb yield

A highly significant ($p < 0.001$) differences were observed among the levels of intra-row spacing and onion varieties on the marketable bulb yield (t/ha). As intra-row spacing increased from 5 to 10 cm, marketable bulb yield in tons/hectare decreased from 34.49 to 28.10. Among the intra-row spacing, a statistically similar result was obtained from 5 and 7.5 cm intra-row spacing, which scored the highest marketable yield in tons per hectare, 34.49 and 32.97, respectively (Table 1). Intra-row spacing of 10 cm showed the lowest (28.1 t/ha). Generally, a trend of increasing gross marketable yield

together with plant density was observed. Plant density has an impact on marketable bulb size and the higher the plant density the smaller the marketable size (Seck and Baldeh, 2009). Kantona et al. (2003) also reported that as plant density increased number of marketable bulbs increased significantly.

The highest marketable bulb yield (34.36 t/ha) was recorded by '*Melkam*' Variety. However, it was not significantly different from '*Bombay*' Red variety. The lowest marketable yield (28.45 t/ha) was recorded on '*Adama*' Red variety, but not significantly different from '*Nasik*' Red (Table 1). In agreement with the present results, Jilani et al. (2009) reported similar observation. A cultivar performs differently under different agro-climatic conditions and various cultivars of the same species grown even at the same environment often yield differently. Thus, performance of a cultivar mainly depends on the interaction of genetic makeup and environment (Jilani and Ghaffoor, 2003).

Average bulb weight (g)

Main effects of intra-row spacing and variety highly significantly ($p < 0.0001$) influenced average bulb weight, but the interaction was not statistically significant. As intra-row spacing increased from 5 to 10 cm, average bulb weight increased from 49.86 to 81.31 g (Table 1). The results are in line with the findings of Rashid and Rashid (1978) who noticed that onion bulb size and weight increases with increasing inter, and intra-row spacing, but recorded lower total bulb yield that increases with closer spacing. Densely populated plants produced lower bulb weight as compared to thinly populated plants. Increasing plant spacing resulted in heavier onion bulbs

Table 2. Effect of intra-row spacing and variety interactions on unmarketable yield of onion.

Intra-row spacing(cm)	Variety	Unmarketable bulb yield (t/ha)
5	Adama Red	2.67 ^a
5	Bombay Red	1.16 ^c
5	Melkam	1.07 ^c
5	Nasik Red	1.68 ^b
7.5	Adama Red	1.01 ^c
7.5	Bombay Red	0.63 ^{def}
7.5	Melkam	0.93 ^{cd}
7.5	Nasik Red	0.88 ^{cde}
10	Adama Red	0.54 ^{ef}
10	Bombay Red	0.32 ^f
10	Melkam	0.53 ^{ef}
10	Nasik Red	0.29 ^f
LSD(0.05)		0.37
CV (%)		26.10

Means in the same column connected with the same letter(s) are not significantly different $p \leq 5\%$ as established by LSD-test.

(Jilani et al., 2009). Mean bulb weight and plant height decreased as population density increased (Mohamedali, 1988). Jan et al. (2003) also found minimum bulb weight at narrower spacing (17 x 4.5 cm).

In the same way, Kantona et al. (2003) reported a decrease in bulb weight as the plant population per square meter increased from 50 to 200 plants likely due to competition associated with closely spaced plants that resulted in lower bulb weight per plant. Abubaker (2008) also reported that the highest yield per plant of bean was obtained from 20 x 30 and 30 x 30 cm planting densities as compared to higher planting densities of 10 x 30 cm. When onions are planted at wider spacing, the emerged shoots get a better microenvironment that resulted in healthy and larger bulbs and high bulb weight per plant. Moreover, better air circulation reduces disease occurrence, which contributes to higher yield per plant. Palada and Crossman (1998) also reported an increase in okra fresh weight per plant from 38 to 70 g with the increase in plant spacing from 31 to 41 cm due to increase in the number of stem and wider leaf area per plant at wider spacing.

'Melkam' variety showed significantly high average bulb weight (75.77 g). The lest value was observed on 'Adama' Red (63.74 g), it was not significantly different from 'Nasik' Red (63.74 g) (Table 1). The lowest average bulb weight (61.03 g) was recorded by 'Adama' Red variety. Difference in average bulb weight within varieties was due to their genetic variability. This finding is in concurrence with the findings of Jilani and Ghaffoor (2003) and Jilani et al. (2009) who reported the variation among onion cultivars in average bulb weight. Kimani et al. (1993) also reported significant bulb weight variation among eight onion cultivars. According the EARO (2004),

'Melkam' variety is characterized by large bulb weight.

Unmarketable bulb yield

The main effect of intra-row spacing, variety and their interaction on unmarketable bulb yield (t/ha) and percentage of unmarketable bulb yield from the total bulb yield showed highly significant ($p < 0.01$) difference. The highest unmarketable bulb yield was produced, by the treatment combination of 5 cm intra-row spacing and 'Adama' Red (2.67 t/ha) followed by treatment combination of 5 cm intra-row spacing and 'Nasik' Red (Table 2). High unmarketable yield in closely spaced plants could be due to inter-plant competition resulting in a fewer large sized bulbs than wider spacing that negatively affected the marketable yield and favored the production of small sized bulbs which are unmarketable. This finding is in agreement with other related reports. Seck and Baldeh (2009) concluded that plant density has an impact on marketable bulb size.

The result further revealed that 'Adama' Red and 'Nasik' Red varieties are relatively less tolerant to narrower intra-row spacing in the study area. In support of the present result, some authors (Rumpel and Felczynski, 1997; Russo, 2008; Jilani et al., 2009; Geremew et al., 2010) also reported similar results that marketable bulb yield and unmarketable bulb yield could be affected by both varietal differences and plant density.

Bulb yield of different size category

Small size bulbs (%)

Highly significant difference was observed for the main

Table 3. Effect of intra-row spacing and variety interactions on percentage of small size and medium size bulbs of onion.

Intra-row spacing (cm)	Variety	Percentage of small size bulbs	Percentage of medium size bulbs
5	Adama Red	23.755 ^a	69.980 ^{cd}
5	Bombay Red	15.449 ^b	75.943 ^{abc}
5	Melkam	10.828 ^{bcd}	77.022 ^{abc}
5	Nasik Red	13.421 ^{bc}	80.514 ^a
7.5	Adama Red	15.112 ^b	75.73 ^{abc}
7.5	Bombay Red	8.626 ^{cde}	75.865 ^{abc}
7.5	Melkam	9.359 ^{cde}	70.328 ^{cd}
7.5	Nasik Red	12.482 ^{bc}	72.974 ^{bcd}
10	Adama Red	6.905 ^e	75.964 ^{abc}
10	Bombay Red	7.203 ^{de}	71.899 ^{cd}
10	Melkam	4.433 ^e	67.897 ^d
10	Nasik Red	7.167 ^{de}	79.388 ^{ab}
LSD(0.05)		5.084	7.45
CV (%)		25.3	6.95

Means with the same letter are not significantly different at $P \leq 5\%$ as established by LSD- test.

effects, intra-row spacing and variety ($p < 0.0001$) and their interaction ($P < 0.01$) on percentage of small size bulb yield. The highest percentage of small size bulbs yield (23.76 2.755) was scored at a combination of intra-row spacing of 5 cm and 'Adama' Red variety (Table 3). It is factual that yield of small bulbs increases with density opposite to the yield of large bulbs, which can be highest at lowest density (Rumpel and Felczynski, 2000). Castellanos et al. (2004) cited in Broome (2009) also reported that super garlic cloves were largest at less dense plantings, while yield of smaller cloves (40 to 45 mm) increased at a higher density. Brewster and Rabinowitch (1990) found that high plant-population density (170 plants/m²), percentage of small bulbs was higher and rates of double bulbs were lower than at low plant density (90 plants/m²).

Medium size bulbs (%)

The interaction effect of intra-row spacing and variety on percentage of medium size bulbs yield showed statistically significant difference ($p < 0.05$). Although, the main effect of variety and intra-row spacing did not show statistically significant difference. The highest medium size bulbs percentage (80.51 80.514) was recorded at a combination of 5 cm intra-row spacing and 'Nasik' Red variety. However, it was not significantly different from 'Melkam' (77.02), 'Bombay' Red (75.94) at 5 cm intra-row spacing, and also 'Bombay' Red (75.87) and 'Adama' Red (75.73) at intra-row spacing of 7.5 cm, and 'Adama' Red (75.96) at 10 cm intra-row spacing.

The lowest medium size bulb percentage was observed at the combination of 10 cm intra-row spacing with 'Melkam' (67.9). However, it was statistically on par with

'Adama' Red (69.98) at intra-row spacing of 5 cm, 'Bombay' Red (71.9) at intra-row spacing of 10 cm, 'Melkam' (70.33) and 'Nasik' Red (72.94) at intra-row spacing of 7.5 cm (Table 3). Nasir et al. (2007) reported maximum weight of medium bulbs (958.50 g/plot) produced at higher planting density of 80 plants/m² and there was parietal difference.

Large size bulbs (%)

The interaction effect of intra-row spacing and variety did not show statistically significant difference, while the main effect of intra-row spacing and variety on percentage of large size bulbs yield showed statistically very high significant ($p < 0.0001$) difference. There was statistically significant difference among all spacing levels.

As the intra-row spacing increased from 5 to 10 cm, the percentage of large size bulbs increased from 9.3 to 20.3 (Figure 1). Likewise, Jilani et al. (2009) reported significant variations for different plant spacing, as the widest spacing showed its superiority over all the other spacing.

Among the varieties, it was revealed that 'Melkam' recorded significantly the highest large size bulbs (20.71), followed by 'Bombay' Red variety. The lowest percentage of large size bulbs was recorded by 'Nasik' Red (11.1) and 'Adama' Red (11.5) (Figure 1). In agreement with this finding, Mallor et al. (2011) reported the difference among onion lines on average bulb size ranging from 150 to 190 g.

Bulb length (cm)

Results (Table 4) show that the effect of the intra-row

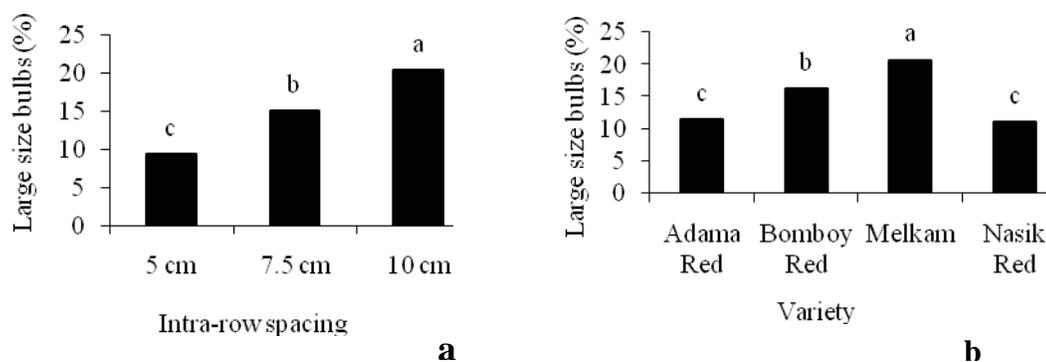


Figure 1. Effect of intra-row spacing (a) and variety (b) on large size bulb percentage of onion. Means followed by the same letter are not significantly different at $p \leq 5\%$ as established by LSD-test (LSD=3.69).

Table 4. Effect of intra-row spacing and variety on bulb length, bulb diameter and bulb neck diameter of onion.

Treatment	Bulb length (cm)	Bulb diameter (cm)	Bulb neck diameter (cm)
Intra-row spacing (cm)			
5	4.11 ^b	4.66 ^c	1.48 ^b
7.5	4.43 ^a	5.23 ^b	1.57 ^b
10	4.54 ^a	5.63 ^a	1.74 ^a
LSD _(0.05)	0.13	0.22	0.12
CV (%)	4.16	6	10.2
Variety			
Adama Red	4.44 ^{ab}	5.07 ^{ns}	1.57 ^b
Bombay Red	4.18 ^c	5.21 ^{ns}	1.54 ^b
Melkam	4.51 ^a	5.2 ^{ns}	1.77 ^a
NasikRed	4.33 ^{bc}	5.11 ^{ns}	1.49 ^b
LSD _(0.05)	0.15	NS	0.14
CV(%)	4.16	6	10.2

NS= Non-significant. Means within a column followed by the same letter(s) are not significantly different according to LSD test.

spacing and variety interaction did not show statistically significant difference, while the intra-row spacing and varieties affected the bulb length of onion significantly ($p < 0.001$). The highest bulb length (4.54 cm) was recorded at the intra-row spacing (10 cm) and intra-row spacing of 7.5 cm (4.43). The narrowest intra-row spacing (5 cm) showed the lowest (4.11 cm) bulb length. As intra-row spacing increased from 5 to 10 cm, bulb length increased from 4.1 to 4.6 cm. The present result is in agreement with those reported by Kantona et al. (2003). Hyder et al. (2007) and Hosmani et al. (2010) also elaborated that there is strong association of bulb length with bulb diameter, and plant height, which were increased as intra-spacing increased.

Significantly highest bulb length (4.51 cm) was obtained from 'Melkam' variety. However, it was not statistically different from 'Adama' Red variety (4.44 cm) (Table 4). In contrast, 'Bombay' Red variety recorded the lowest (4.18 cm), but it was on par with 'Nasik' Red

variety (4.33). This is due to the reality that varieties can have different characteristics like bulb shape and color as suggested by Lemma (2004). Jilani et al. (2009) also found difference among cultivars in bulb length due to genetic inheritance.

Bulb diameter (cm)

There was no statistically significant difference of interaction of variety and intra-row spacing on bulb neck diameter. Meanwhile, the main effect of intra-row spacing showed statistically very highly significant ($p < 0.0001$) difference in bulb diameter. Intra-row spacing of 10 cm showed the highest bulb diameter (5.63 cm), followed by 7.5 (5.23 cm) and 5 cm (4.66 cm) intra-row spacing (Table 4). The increase in bulb diameter at wider intra-row spacing could be probably attributed to more nutrients space and moisture availability. Bulb diameter

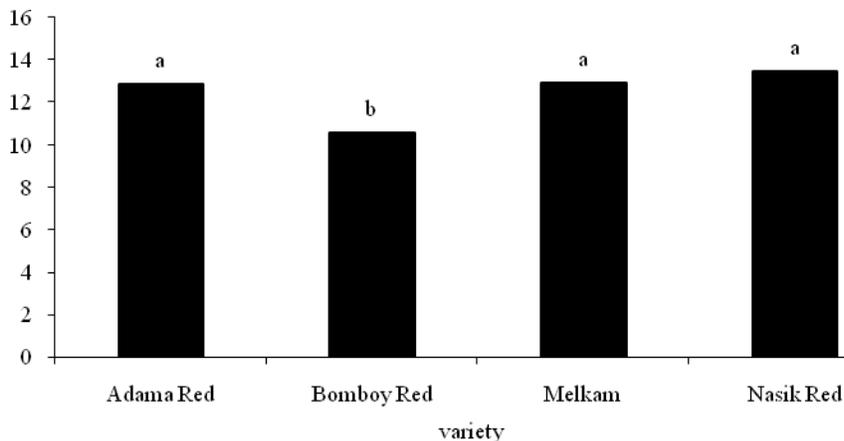


Figure 2. Differences in the percentage of bulb dry matter content (%) of onion varieties. Means followed by the same letter are not significantly different at $p \leq 5\%$ as established by LSD-test (LSD=1.46).

contributes significantly to yield component of a crop (Cheema et al., 2003; Jilani and Ghaffoor, 2003). The present findings are in line with those reported by Rashid and Rashid (1978), Quadir and Boulton (2000), Jan et al. (2003), Kantona et al. (2003), Akoun (2005), Hyder et al. (2007) and Jilani et al. (2009). Mohammedali (1988) also reported wider intra-row spacing gave larger bulbs of onion. Tendaj (2005) reported an increase in intra-row spacing of shallot from 5 to 20 cm, resulting in increment of percent share of bulbs having greater than 25 mm diameter from 13.70 to 47.20%.

Bulb neck diameter (cm)

Thickness of neck is an important parameter that determines the storability qualities of onion varieties. The results indicated that the main effect of both intra-row spacing and variety on bulb neck diameter (cm) showed significant ($p < 0.001$) difference; the interaction had no significant effect on bulb neck diameter. The highest bulb neck diameter (1.74 cm) was observed at the intra-row spacing of 10 cm (Table 4). The least bulb neck diameter (1.48 and 1.57 cm) was recorded at intra-row spacing of 5 and at 7.5 cm intra-row spacing, respectively. The current finding complies with the work of Brewster and Rabinowitch (1990). In agreement with this result, Jilani and Ghaffoor (2003), Kantona et al. (2003), Jilani et al. (2009) and Khalid (2009) reported similar results.

'Melkam' showed significantly the highest bulb neck diameter (1.77 cm), while there was similar results in the rest varieties (Table 4). The presence of variation in bulb neck diameter among onion cultivars is also reported by Mar (1994), Kalb (2001), Jilani and Ghaffoor (2003) and Jilani et al. (2009). According to Currah and Proctor (1990), thick neck in onion is caused by the onion growth actively in that the neck did not become dormant and

resulted to undifferentiated scales with high thickness at wider inter plant spacing. This indicated that thick neck in onion causes delay in bulbing and has a negative impact on bulb yield, especially when water stress might be encountered lately. Other workers such as Brewster and Rabinowitch (1990) as well as Lemma and Shimeles (2003) explained that the cause of thick neck in onion is generally due to defective nutrition prolonged cool time and lack of interplant competition in addition to genetic inheritance.

Bulb dry matter content (%)

Intra-row spacing effect and interaction effect did not show significant difference. However, varieties had significant ($p < 0.01$) effect on bulb dry matter content. In conformity with this result, Mohamodali (1988) and Abubaker (2008) reported that different in-row spacing seems to have no effect on the dry matter content. The lowest percentage of dry matter content (10.6) was recorded by 'Bombay' Red variety, while there is statistically high and similar results shown on the other three varieties (Figure 2). The result is consistent with the findings of Islam et al. (2007), Magdi et al. (2009), Mousa and Mohamed (2009) who reported significant difference among onion genotypes in dry matter content. Varieties known as 'storage onions' have high dry matter content (15 to 20%) and relatively high amounts of fructans, but have lower levels of reducing sugars, besides relatively high rates of organosulfur compounds (Mallor et al., 2011).

Kimani et al. (1993) additionally reported a dry matter content variation from low levels of 7 to 10% to high levels of 15 to 20% in onion varieties. The authors suggested that onions with high dry matter are preferred for processing. They further showed that onions with high

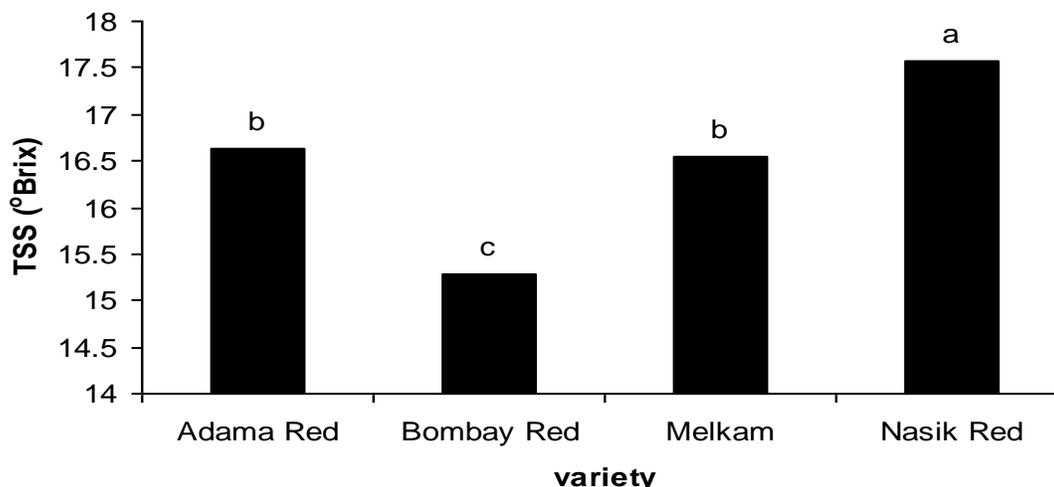


Figure 3. Differences in the TSS content ($^{\circ}$ Brix) of onion varieties. Means followed by the same letter are not significantly different at $p \leq 5\%$ as established by LSD-test (LSD=0.55).

dry matter content tend to low yield than those with low dry matter content and the latter exhibit rapid bulbing that contradicts the high dry matter content found in '*Melkam*' variety while having high yield. The range of dry matter content of the varieties is similar to previous studies done in Ethiopia, which stated that dry matter content in bulb onion varies in according to cultivars. It varied from low level of 7 to 10% to high level of 15 to 20% and average 11 to 15% (Lemma and Shimeles, 2003). The authors further explained that the lower levels are usually rapidly bulbing, becoming soft texture and usually with low keeping qualities. The higher levels of dry matter in onion found in selected cultivars are important for dehydration.

Total soluble sugars content (TSS)

Effect of intra-row spacing and interaction did not show statistically significant difference on TSS content of onion while varieties had significant ($p < 0.0001$) effect on total soluble sugar (TSS) content of onion bulb.

In this experiment, '*Nasik*' Red gave significantly the maximum TSS content (17.6 $^{\circ}$ Brix), followed by '*Adama*' Red and '*Melkam*' varieties (Figure 3). However, there was no significant difference between the latter two varieties. '*Bombay*' Red showed significantly the minimum TSS content (15.3 $^{\circ}$ Brix) (Figure 3). High total soluble solids (TSS) have been proved to be associated with long storage life (AVRDC, 2000). Cultivars with high bulb yields may have lower total soluble solids content as compared to the cultivars with lowest yields. This observation is also in line with the report of EARO (2004) which described '*Bombay*' Red variety with low TSS in $^{\circ}$ Brix than the other varieties of onion released and being cultivated in Ethiopia. Varieties used for storage have high and intense, pungent flavour and are desirable

characteristics for cooking or for industrial processing (Mallor et al., 2011). Hosmani et al. (2010) suggested that there could be less influence of environments for these traits.

Rajcumar (1997) also reported that a total soluble solid variation of about 4.0 to 16.3% could exist among different cultivars of onion. The author further explained his findings that cultivars with high bulb yields have lower total soluble solids content as compared to the cultivars with lowest yields and a negative correlation ($r = -0.85$) between bulb yield and soluble solids content was found, suggestive of a strong association between these two characters.

Mallor et al. (2011) also reported significant negative correlation between bulb weight and soluble solids content. They elaborated that these results indicate a trend in larger onions which contain lower rates of both organo-sulfur derivatives and carbohydrates; therefore, suggesting that bulb size increase was because of higher water content. Cultivars with high bulb yields have lower total soluble solids content as compared to the cultivars with lowest yields (AVRDC, 1990, 2008).

Summary and conclusion

Onion is one of the popular and the most cultivated vegetables in Ethiopia in general and in Tigray region in particular. Farmers in the study area produce onion as a cash crop using non-uniform plant spacing based on the existing indigenous knowledge.

The study was conducted to investigate best plant spacing for highest yield and better quality of onion varieties and to recommend best variety adaptable to the specific area and best plant spacing that give best yield and bulb quality.

The experiment was conducted from August 2010 to April 2011 under irrigated condition at Aksum area, L/maichew district, Central Zone of Tigray National Regional State. The experiment was done at three intra-row spacings (Factor 1): S1 (5 x 20), S2 (7.5 x 20) and S3 (10 x 20) cm equivalent to densities of 90, 67 and 45 plants/m²; respectively, and four onion varieties (Factor 2): 'Adama' Red, 'Bombay' Red, 'Melkam', 'Nasik' Red. A 3 x 4 factorial experiment was laid out in RCBD with four replications. Data were collected on yield and quality parameters.

Results of the study show that main effects of intra-row spacing, varieties as well as their interactions had considerable influence on different parameters. The highest total bulb yield (36.14 t/ha) was recorded at intra-row spacing of 5 and by 7.5 cm (33.82 t/ha).

Highest total bulb yield (35.2 t/ha) was also recorded on 'Melkam' variety, while the lowest yield (29.86 t/ha) was recorded on 'Adama' Red variety. Intra-row spacings of 5 and 7.5 cm also had higher marketable yield than 10 cm. As intra-row spacing increased from 5 to 10 cm average bulb weight in grams increased from 49.86 to 81.31 g.

The highest average bulb weight (75.77 g) was recorded on 'Melkam' variety followed by 'Bombay' Red (67.29), while the lowest average bulb weight (61.03 gm) was recorded by 'Adama' Red variety. The highest unmarketable yield was produced, at the combination of 5 cm intra-row spacing and 'Adama' Red variety (2.67 ton/ha). The highest percentage of small size bulbs (23.76 and 15.45%) was produced by the treatment combination of 'Adama' Red at 5 cm spacing and 'Bombay' Red at 5 cm spacing, respectively.

While the minimum percentage of small size bulbs (4.4 and 6.9) was found in the combination of 'Melkam' at 10 cm and 'Adama' Red at 10 cm spacing, respectively. The highest percentage of large size bulbs (20.71) was recorded in 'Melkam' variety, while the lowest percentage of large bulbs (11.1) was obtained in 'Nasik' Red variety. The finding suggested that it is better to use intra-row spacing greater than 5 cm to minimize more small bulbs as this is not mostly preferred for market.

Besides, the ultimate goal of onion production is profitability through yield enhancement; the result revealed that 'Melkam' and 'Bombay' Red varieties appeared to be superior for yield and earliness at the study area although it needs repeated research for complete recommendation.

The highest percentage of bulb dry matter content (13.47) was recorded on 'Nasik' Red variety, while the lowest percentage (10.6) was recorded on 'Bombay' Red variety. The highest TSS in °Brix, was found at the late matured varieties 'Nasik' Red (17.57), while the lowest TSS in °Brix was found at the early varieties 'Bombay' Red (15.29) followed by 'Melkam' (16.54).

Hence, for fresh consumption, the milder ones with lesser TSS in °Brix value are better for use since they

have better yield and adaptability advantages at the dryland condition of Tigray in general and Aksum area in particular.

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