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African Journal of Plant Science

Full Length Research Paper

Effects of different hormones on organogenesis *in vitro* of some varieties of cassava (*Manihot esculenta* CRANTZ) grown in Senegal

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Cassava (*Manihot esculenta*) is a perennial euphorbiaceous shrub grown mainly for its starchy tubers and its leaves rich in protein. The most known method of propagation of this crop is the classical cuttings' planting. However, *in vitro* propagation appears most useful and permits to obtain high quantity of healthy vegetable material in a short period. In this work, it was to study the impact of different hormones on the organogenetic capacities *in vitro* of five cassava varieties cultivated in Senegal. Axillary uninodal sections were disinfected and cultured in Murashige and Skoog (MS) basal medium added or not of different concentrations (0.1, 0.5 and 1 mg/L) of auxin (α-naphthalene acetic acid (NAA)) or cytokines (benzyl aminopurine (BAP) and kinetin). Best shoot growth and rooting was observed in MS medium containing 0.1 mg/L kinetin with normal development of the leaves. Highest proliferation of shoots and leaves was obtained with medium MS + BAP 1 mg/L. Callus formation was observed in all media containing hormone but most in MS + NAA 1 mg/L. This work proposes a rapid and economic technique for cassava multiplication.

Key words: Organogenesis in vitro, cassava, varieties, hormones, Senegal.

INTRODUCTION

Manihot esculenta known as cassava is a plant-tubers grown mainly in tropical regions where it presents a high economic importance. According to FAO (1995) it is the most important locally-produced food in a third of the world's low-income and food-deficit countries, especially in Africa. Its starchy roots and high-protein leaves are consumed at home or sold, in fresh or in processed form. Because of its simple technology of culture and large flexibility, cassava is often grown in rural areas where other crops fail (Thro et al., 1998).

However, cassava propagation using cuttings classical method is not adequate for rapid and healthy

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multiplication. The annual production of vegetable material of this plant is very low (average 10 cuttings per plant per year) and yields are reduced by pests and diseases attack, essentially by the cassava african mosaic, the cassava bacterial blight due to *Xanthomonas campestris* and the floury cochineal (*Phenacoccus manihotis*).

An alternative method that proves to remediate the low coefficient of multiplication and infections of cassava is micropropagation *in vitro*. It consists of regenerating whole plants by cultivating explants in artificial medium, sterile conditions and controlled environment (light and temperature). Several plants conserving genetic identity are obtained from successive generations and can be multiplied to infinity. According to Guo and Liu (1995), hundreds of millions of plants derived from culture *in vitro* are produced annually in the world.

Micropropagation is therefore a rapid and powerful technique of multiplication that permits to obtain plants in sufficient quantity to satisfy continuous production independent of the seasons. Indeed, contrarily to the classical method that gives a single individual by seed or cutting, *in vitro* propagation may produce as many copies as liked from a single explant (Rancillac, 1981) and help to overcome constraints related to the availability of high quality of planting material (Wheatley et al., 2005; Vaillant et al., 2005).

The work of Fereol (1978) highlighted perfectly the interest of culture *in vitro* in cassava. The ability of vegetative propagation *in vitro* has been estimated in african clones, to a potential of production of a million plants a year from a single cutting (Lourd, 1981). Thus, with this method of propagation, research can make a significant contribution to food security and economic development in the areas of culture of cassava (Thro et al., 1998). This contribution is more interesting when it comes to propagate resistant or tolerant varieties to major constraints to the culture.

In this present study, it was made to study the effect of some hormones on organogenetic capacities *in vitro* of 5 varieties of cassava grown in Senegal and selected for their tolerance to termites ravaging cuttings *Odontotermes* sp. aff. *erraticus* (Faye et al., 2014). The general objective of the study was to determine the best medium for an optimal response of cassava to multiplication *in vitro*. Specific objectives were to assess the effects of different hormone concentrations on the growth and development (caulogenesis, rooting, shoot and root growth, phyllogenesis and callus formation) *in vitro* of the different varieties of cassava.

MATERIALS AND METHODS

Plant material

The plant material was composed of five cassava varieties cultivated in the Department of Tivaouane (Senegal) including 3 local: *Soya*, *Cololi* and *Niargi* and 2 Brazilian: *Cacau* and *Cacau roja*. Cuttings were collected from farms and transplanted into pots placed in a greenhouse. They were watered every two days at 1/2 L per cutting. Fungicide and insecticide treatments using respectively 50 mg/L mancozeb and 2 ml/L chlorpyrifos-ethyl were conducted in this culture. Plants obtained have provided explants which were used for material of introduction *in vitro*.

Sterilization

After a brief wash with soap followed by 3 successive rinses, explants have been soaked for 20 min in a solution of 80 g/L calcium hypochlorite with 2-3 drops of tween 20, then in bleach for 10 min. Each soaking was immediately followed by 3 successive rinses with sterile water.

Culture media and control

The basal nutrient used was the complete MS (Murashige and Skoog) (1962) medium. It was solidified with 8 $g.L^{-1}$ agar at pH adjusted 5.7 and contained 25 $g.L^{-1}$ of sucrose. This medium was or not supplemented with NAA (α-naphthalene acetic acid), BAP (benzyl aminopurine) or kinetin at different concentrations (0.1, 0.5 and 1 mg/L). The media were distributed in glass test tubes at 20 ml/tube and then sterilized by autoclaving at 110°C for 20 min. The surface disinfected explants were placed vertically on the media, in sterile conditions. Cultures were incubated in the dark for 24 h and then transferred to the culture chamber lighted with 4000 lux where they were maintained at 27 ± 1°C, under a photoperiod of 13 h day.

A sample of 12 tubes per medium per variety was considered for the parameters assessment. Weekly measures have been conducted on the 3rd generation plantlets during 30 days of culture. The parameters evaluated were the shoot and root numbers and growth, the number of leaves and the rate of callus formation.

Statistical analyses

Data collected on this study were entered on Excel and analyzed with software Costat. They have been subjected to analysis of variance of the Student, Newman and Keuls test at the 0.05 level of significance.

RESULTS

Effect of hormones on caulogenesis *in vitro* of the different varieties of cassava

Figure 1 shows that the addition of hormones in the basal medium MS favored multiple shoots formation in all the varieties of cassava. Indeed, average 5.3 shoots by plantlet were count in the variety *Soya* in the MS + BAP 0.1 mg/L medium after four weeks of culture. Highest numbers of shoots were observed in medium containing BAP or kinetin. Medium with NAA were however less favorable to caulogenesis. The variation of shoots number were significant (F = 0.383; P = 0.05) among the different hormonal concentrations experimented, according to statistics.

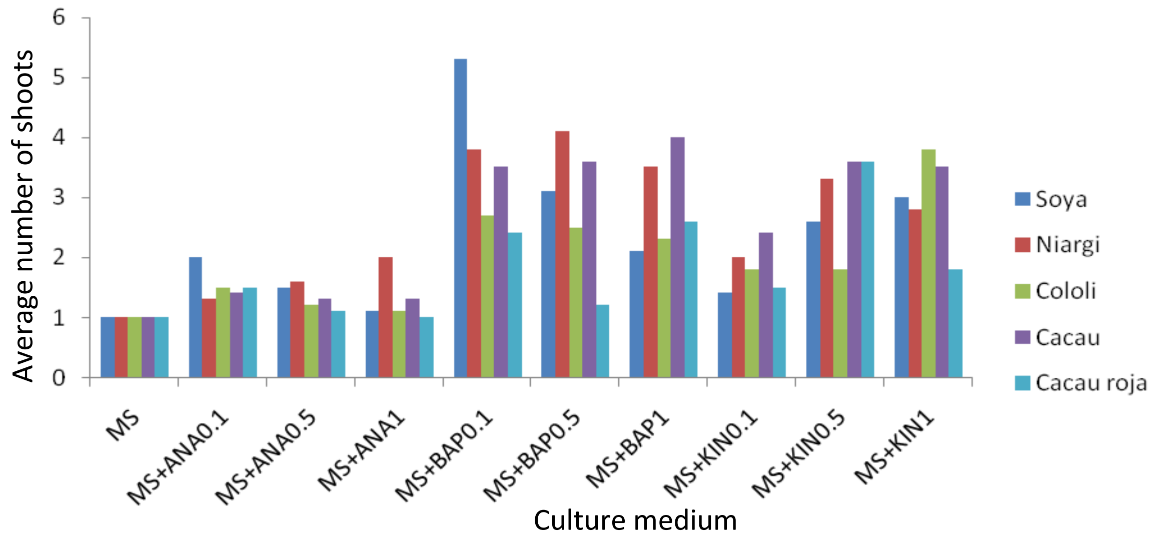

Figure 1. Influence of different hormonal concentrations (mg/L) and control on shoots' proliferation *in vitro* of 5 varieties of cassava after 1 month of culture.

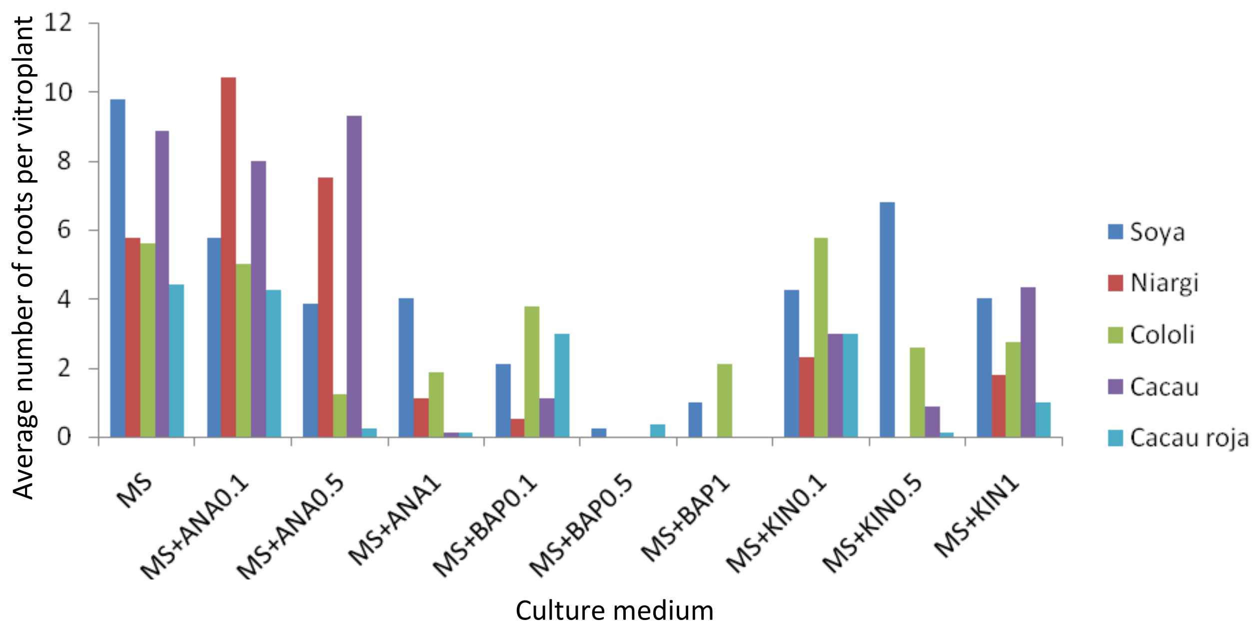

Figure 2. Influence of different hormonal concentrations (mg/L) and control on rooting *in vitro* of 5 varieties of cassava after 1 month of culture.

Effect of hormones on rooting *in vitro* of the different varieties of cassava

The extent of root formation *in vitro* in cassava depends on the culture medium. We noted that the addition of BAP, especially at high doses in the MS medium reduced strongly the formation of roots. Indeed, no rooting was observed among the varieties *Niargi*, *Cacau* and *Cacau roja* in the MS + BAP 1 mg/L medium during 1 month of culture. In contrast, highest numbers of roots (average 10.4 per plantlet in variety *Niargi*) were found in MS + NAA 0.1 mg/L after 4 weeks of culture (Figure 2). According to ANOVA, the number of roots in the 5 varieties of cassava varied significantly (F = 3.453; P =

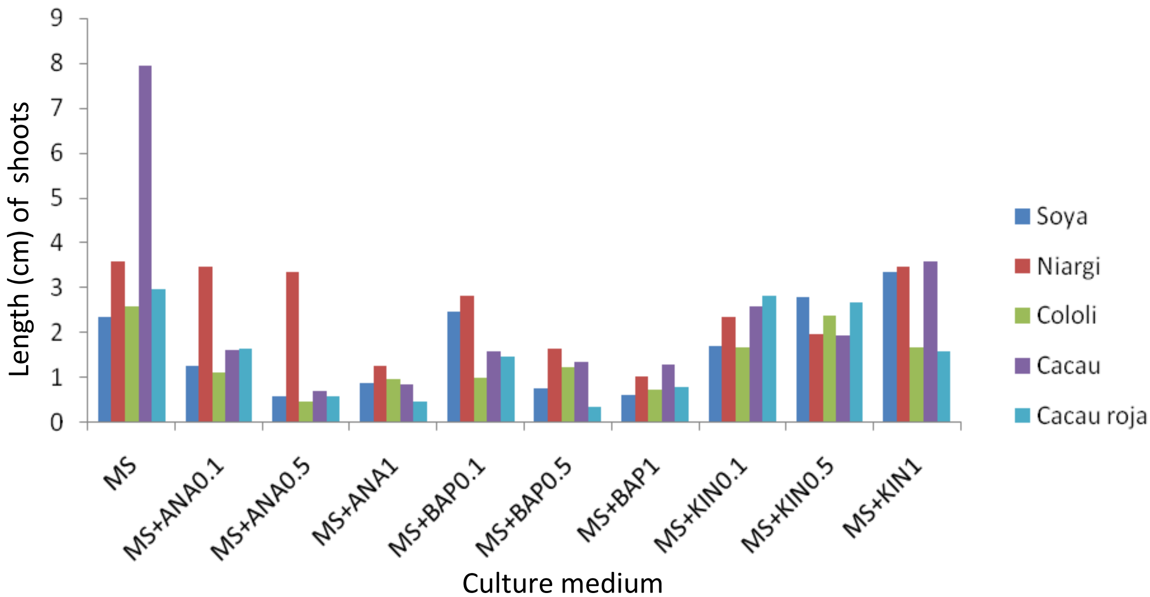

Figure 3. Influence of different hormonal concentrations (mg/L) and control on shoots' growth *in vitro* of five varieties of cassava after 1 month of culture.

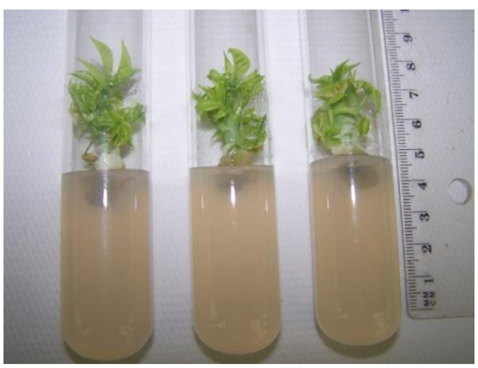

Figure 4. Poor shoots growth in MS+BAP 0.1 mg/L after 30 days of culture.

0.05) among the hormonal concentrations tested.

Effect of hormones on shoots' growth *in vitro* of the different varieties of cassava

Explants cultured in media containing kinetin showed best growth of shoots. Respectively 3.56, 3.44 and 3.33 cm averages of shoots' lengths were observed among varieties *Cacau*, *Niargi* and *Soya* in MS + KIN 1 mg/L after four weeks of culture (Figure 3). However BAP and NAA were less favorable to shoots' growth. Therefore shortest shoots with averages 0.45 and 0.32 cm long, respectively have been observed among varieties *Cololi* and *Cacau roja* in the media MS + NAA 0.5 mg/L and MS + BAP 0.5 mg/L after 1 month of culture.

According to the analysis of variance, the variation of the shoots' length among the different culture media tested was significant (F = 0.639; P = 0.05).

Effect of hormones on roots' growth *in vitro* of the different varieties of cassava

The elongation of plantlets' roots varied depending on the culture medium. Thus, we have seen that the addition of hormone, especially in high concentrations, in the medium MS enhanced the roots growth. Kinetin proved however best elongation of roots in some varieties. Thus, we could record respectively 4.62 and 3.58 cm averages of roots' lengths among varieties *Cacau* and *Soya* in the MS + KIN 1 mg/L medium after 1 month of culture. In contrast, the lowest root growth occurred in media containing the NAA where very short roots not exceeding 0.06 cm average have been observed in the variety *Cacau roja* in the medium MS + NAA 1 mg/L after 4 weeks of culture (Figure 5). The variation of the roots' length was significant (F = 1.554; P = 0.05) among the culture media experienced, according to the ANOVA.

Effect of hormones on the phyllogenesis *in vitro* of the different cassava varieties

Figures 4 and 7 show that explants cultured in medium

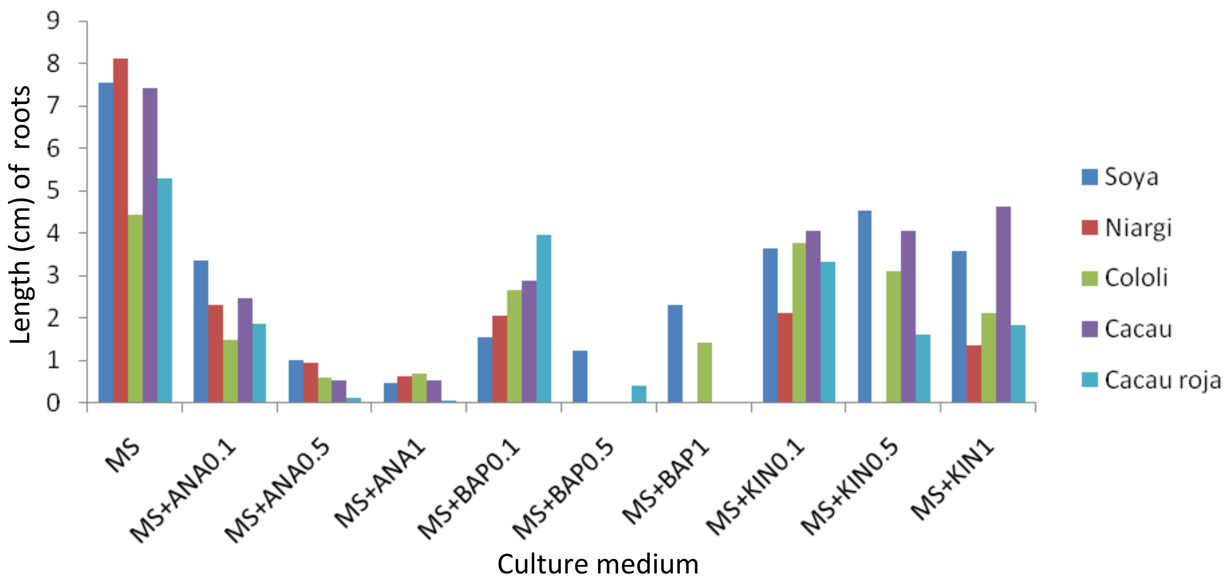

Figure 5. Influence of different hormonal concentrations (mg/L) and control on roots' growth *in vitro* of five varieties of cassava after one month of culture.

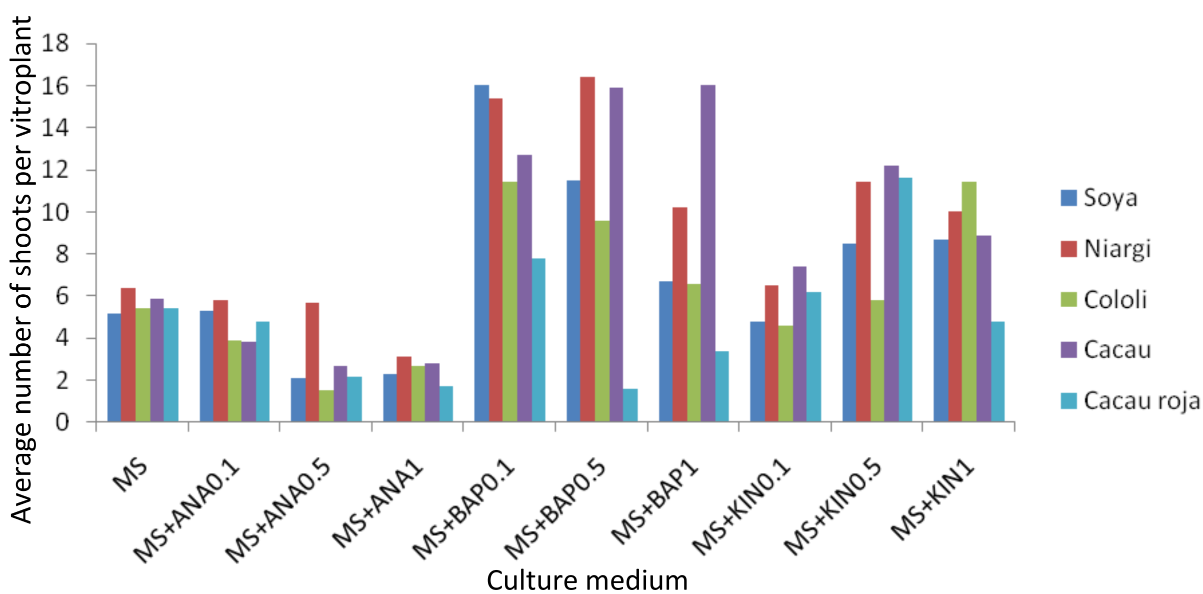

Figure 6. Influence of different hormonal concentrations (mg/L) and control on phyllogenesis *in vitro* of five varieties of cassava after 1 month of culture.

with BAP produced more leaves than those cultured in medium containing kinetin. Indeed, after four weeks of culture we could count up to 16.4 leaves average in plantlets of the variety *Niargi* in the MS + BAP 0.5 mg/L medium. Leaves produced in media addition of kinetin presented however the best development. In contrast, NAA appeared to be a hormone unfavorable to the phyllogenesis in all varieties. Thus the number of leaves per plantlet could fall up to 1.5 average after 4 weeks of culture in the medium MS + NAA 0.5 mg/L in the variety *Cololi* (Figure 6).

The number of leaves formed *in vitro* was significantly variable (F = 10.310; P = 0.05) among the different culture media tested, according to the analysis of

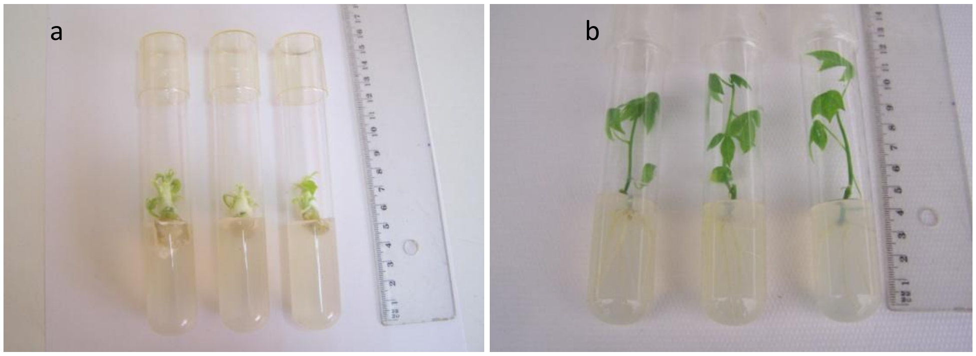

Figure 7. Shoots growth in MS+ANA0.1 mg/L (a) and MS+KIN0.1 mg/L (b) media after 30 days of culture.

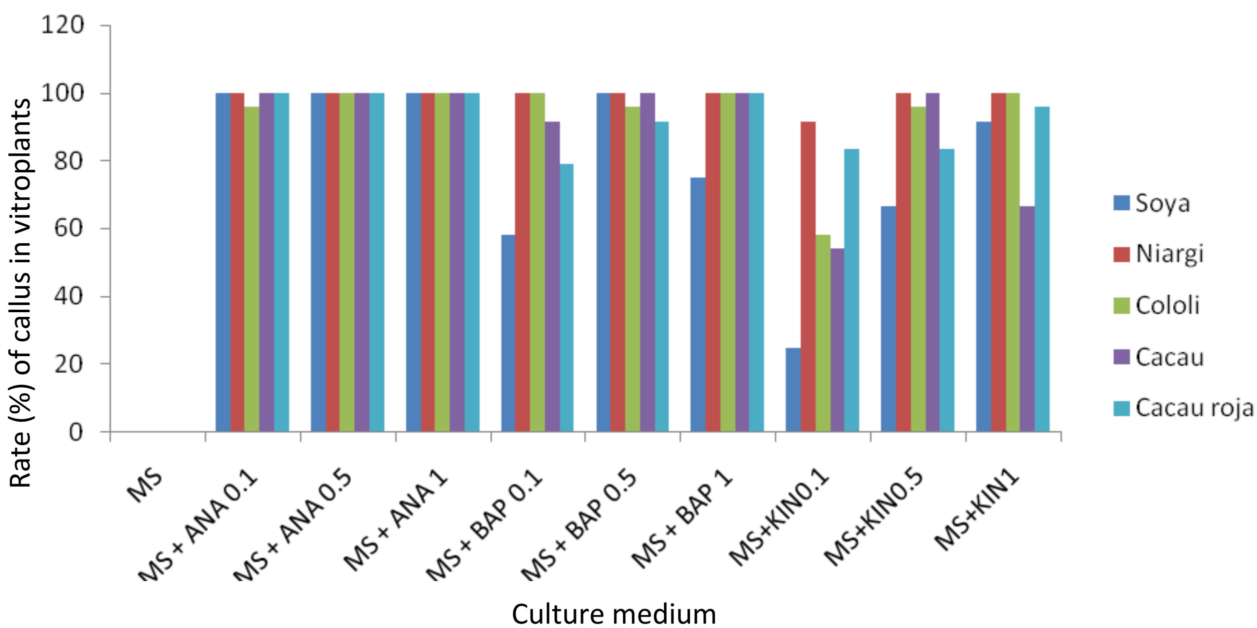

Figure 8. Influence of different hormonal concentrations (mg/L) and control on callus formation *in vitro* of five varieties of cassava after one month of culture.

variance.

Effect of hormones on the callus formation *in vitro* of the different varieties of cassava

Callus formation in plantlets appeared to be induced by the addition of growth regulators in the basal medium MS. Figure 8 shows that NAA, especially at high concentration, were more favorable to the callus formation, compared to the BAP and the kinetin which had the least. Indeed, after 4 weeks of culture, we recorded 100% of callus formation in all varieties in media MS + NAA 0.5 mg/L and MS + NAA 1 mg/L against 25 and 58.3%, respectively in varieties *Soya* and *Cololi* in the MS + KIN 0.1 mg/L medium. No callus formation was observed however in the basal medium MS (Figure 4). The analysis of variance showed that the callus formation's rate varied significantly (F = 32.538; P = 0.05) among the different media tested.

DISCUSSION

Our results showed that the response of cassava to micropropagation *in vitro* depends on the culture medium.

Thus, the addition of growth regulators at different concentrations in the basal culture medium MS oriented the organogenesis *in vitro* of this plant. In our experimental conditions, we could note that the application of a low concentration (0.1 mg/L) of NAA, BAP or kinetin favored more than high concentration (1 mg/L), best growth of organs except callus. Such observations are confirmed by the results obtained by Cacai et al. (2012) according to which the effect of kinetin on the shoots' growth varies depending on the applied concentration. Das et al. (2013) also found that the nature and the concentration of cytokinin determined significant growth' variations in some genotypes of Dioscorea sp. ; reflecting the results of Ondo et al. (2007) who, using high concentration (2 mg/l) of kinetin, have noticed a reduction of the length of roots in the *Dioscorea cayenensis – Dioscorea rotundata* complex.

In our experiment, explants cultured in media containing BAP produced highest numbers of shoots and leaves in all the cassava varieties. Kinetin resulted better growth of shoots, roots and leaves, while NAA induced more importantly the formation of callus, compared to the 2 cytokinins (BAP and kinetin). These results are confirmed by the works of Malaurie et al. (1995), Miller and Skoog (1957), James and Newton (1977), Navatel (1979), Ammirato (1984), Bennett et al. (1986), Lalmohanlal et al. (1990), Romano et al. (1992), Boniface (1992), Yepez et al. (2001), Kbiach (2002), Namwenje et al. (2003) and Ahanhanzo et al. (2008) reported by Cacai et al. (2012), which have shown that kinetin induces more roots than BAP. Indeed, these cytokinins, unlike auxin (NAA), would encourage more development and growth of the aerial organs (shoots and leaves) than of roots. Therefore, according to Phil et al. (1995) the variations of the ratio auxin/cytokinin have specific effects on the development of the explants and then determine the organogenesis tendency. These authors have shown that a high ratio (more auxin than cytokinin) resulted in differentiation of roots, while a low ratio (more cytokinin than auxin) resulted in differentiation of shoots. However, Ahanhanzo et al. (2008), using NAA (0.5 mg/l) + BAP (0.5 mg/l) on one hand and NAA (0.5 mg/l) + KIN (0.5 mg/l) on the other hand, have not observed callus formation in three varieties of cassava (RB 89509, BEN 86052 and TMS 30572). This would be explained by the fact that there was some interaction between growth regulators so that shoot or root differentiation would depend on the type of hormonal combination made.

Our results also show that the organogenesis *in vitro* of the different varieties of cassava were ideal in the culture medium MS, without any addition of hormone. Plantlets were well rooted and showed good vegetative development in this basal medium in all varieties. Such findings agree with results obtained by Boher (1988) and Lourd (1981) which could successfully multiply 65 cassava cultivars without using growth regulator. It appeared according to this researcher that the plantlets' growth *in vitro* could however be extremely variable depending on the cultivars or the type of explants of the same cultivar.

Therefore, according to our results, the variety would also have an influence on organogenesis *in vitro* of cassava. Thus, varieties *Soya*, *Cacau* and *Niargi* have presented best growth and development of organs, compared to varieties *Cololi* and *Cacau roja* in the culture medium MS. Such observations are confirmed by the work of Cacai et al. (2012) according to which the reaction of explants in different culture media is not the same from one variety to another. According to these authors, MS + KIN and MS + NAA media gave the highest shoots' length average in beninese cultivars *Agric Sazoue* (3.54 ± 0.4 cm) and *Gbeze* (6.36 ± 0, 3 cm) on one hand and *Ahouandjan* (8.62 ± 0.8 cm) on the other hand respectively.

In varieties *92/0057* (1.63±0, 1 cm), *BEN 86052* (4.20 ± 0.6 cm), *Sekandji* (6.6 ± 0.4 cm) and *Okoyao* (1.85 ± 0.3 cm) however, it was the NAA + KIN combination that gave the highest averages of shoot length. Similarly, work conducted by Ahanhanzo et al. (2010) on different genotypes of yam have confirmed that the response of the microcuttings to cytokinins' action depended on the genotype of the plant.

Conclusion

Organogenesis *in vitro* of cassava explants depends on the culture medium and the variety. The addition of different growth regulators (NAA, BAP and kinetin) at different concentrations (0.1, 0.5 and 1 mg/L) each in the basal Murashige & Skoog (MS) medium allowed to observe different tendencies in organs' development among 5 varieties *Soya*, *Niargi*, *Cololi*, *Cacau* and *Cacau roja* put in experience. The cytokinin BAP enabled to obtain more shoots, more leaves and fewer roots, while NAA has the most promoted callus formation in all varieties. Kinetin was found to be more favorable to best elongation of shoots and roots and the normal development of the leaves. In all media, rooting and growth of shoots and roots, conversely to number of shoots as well as the phyllogenesis and the callus formation, were more favored with low (0.1 mg/L) than with high (1 mg/L) hormonal concentration. However, the ideal response was observed with the basal culture medium MS in all the cassava varieties, so that the use of hormones appears not necessary for propagation *in vitro* of this plant.

Conflict of Interest

The authors have not declared any conflict of interest.

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REFERENCES

Ahanhanzo C, Agbangla C, Agassounon DTM, Cacaï GHT, Dramane K (2008). Comparative study of the influence of growth' regulators on morphogenesis *in vitro* of some cassava varieties (*Manihot esculenta*) in Benin. Rev. CAMES – Ser. A, 07:47-52.

Ahanhanzo C, Gandonou C, Agbidinoukoun A, Dansi A, Agbangla C (2010). Effect of two cytokinins in combination with acetic acide α-naphthalene on yams (*Discorea* spp.) genotypes response to *in vitro* morphogenesis. Afr. J. Biotech. 9(51):8837- 8843.

Ammirato PV (1984). Yams. In Handbook of Plant Cell Culture (vol. 3). Crop Species Macmillan; New York: 336-354.

Bennett LK, Davies FTJ (1986). *In vitro* propagation of *Quercus shumardii* seedlings. Hort. Sci. 21(4):1045-1047.

Boher B (1988). Cassava healthy material production by *in vitro* culture. Plant Cell Tissue and Orqan Cidture. Agron. 6(3):221-228.

Cacai GHT, Ahanhanzo C, Dangou JS (2012). Effect of different hormonal combinations on organogenesis *in vitro* of some local improved varieties of cassava (*Manihot esculenta-Euphorbiaceae*) cultivated in Benin. Int. J. Biol. Chem. Sci. 6(4):1593-1607.

Das S, Dutta MC, Mazumdar PB (2013). Micropropagation of *Dioscorea alata* L. through nodal segments. Afr. J. Biotech. 12(47):6611-6617.

FAO/GIEWS (Global Information and Early Warning System on Food and Agriculture) (1995). Food supply situation and crop prospects in sub-Saharan Africa. Special Africa Report, April 1995.

Faye A, Kane PD, Sall-Sy D, Sane D, Mbaye DF (2014). Study of the cassava varietal sensitivity to termites ravaging cuttings planted in farms in the department of Tivaouane (Senegal) Int. J. Sc. Adv. Tech. 4(6):6-16.

Fereol C (1978). Vegetative multiplication and elimination of the cassava mosaic disease by thermothérapy inplants cultivated *in vitro*. Diseases of Tropical Food Crops. Int. J. Corpus Linguist. 65(7):285-295.

Guo JY, Liu YQ (1995). Rapid propagation of cassava by tissue culture and its application in rural districts in China. In Proceedings of the Second International Scientific Meeting, Cassava Biotechnology Network, Bogor, Indonesia, 25-28 Aug. 1994, *CIAT* Working Doc. N°. 150:183-189.

James DJ, Newton B (1977). Auxin-cytokinin interactions in the *in vitro* micropropagation of strawberry. Acta Hort. 78:321-331.

Kbiach ML, Lamart A, Abdali A, Badoc A (2002). *In vitro* buds' culture of the oak-cork (*Quercus suber*). Bull. Soc. Pharm. Bordeau 141:73-78.

Lourd M (1981). Cultivation *in vitro* of a collection of cassava variéties. Balance after 4 months of experimentation. ORSAY, 1981.

Miller CO, Skoog F (1957). Chemical regulation of growth and organ formation in plant tissues cultured *in vitro*. Symp. Soc. Exp. Biol. 11:118-130.

Murashige T, Skoog F (1962). A revised medium for rapid growth and bio assays with tissue culture. Physiol. Plant. 15:473-497.

Nweke F (1996). Processing Potential for Cassava Production Growth in Sub- Saharan Africa. COSCA Working. Collaborative Study of Cassava in Africa, IITA Ibadan, Nigeria; Paper N° 11.

Ondo OP, Kevers C, Dommes J (2007). Bud an tuber proliferation *in vitro* of Dioscorea cayenensis-D. rotundata complex. Plant Cell. Tiss. Organ Cult. 91:107-114.

Rancillac M (1981). Making a vegetative multiplication *in vitro* method of the maritime pine (Pinus pinaster Sol.) for clones constitution by seeds, AFOCEL, pp. 41-48.

Romano A, Noronha C, Martins-Loução MA (1992). Influence of growth regulators on shoot proliferation *in vitro* of Quercus suber L. Ann. Bot. 70(6):531-536.

Saleil V, Degras L, Jonard R (1990). Obtention of plants without virus of the yam mosaic (YMV) by *in vitro* culture of apex in american yam Dioscorea trifida L. Agronomy Elsevier/ INRA 10:605-615.

Sane D, Borgel A, Chevallier MA, Gassama-Dia YK (2001). *In vitro* induction of Acacia tortilis subsp. raddiana micro-cuttings' rooting by transitory auxin treatment. Ann. For. Sci. 58:431-437.

Thro AM, Taylor N, Raemakers K, Visser R, Puonti-Kaerlas J, Schopke C, Iglesias C, Roca W, Sampaio MJ (1998b). Biotechnology and the cassava farmer: an agenda to make a difference. Nat. Biotech. 16:428-430.

Vaillant V, Bade P, Constant C (2005). Photoperiod affects the growth and development of yam plantlets obtained by *in vitro* propagation. Biol. Plant. 49:355-359.

Wheatley AO, Ahmed MH, Asemota HN (2005). Development *in vitro* and salt adaptation of greater yam (Dioscorea alata) plantlets. *In vitro* Cell. Dev. Biol. Plant. 39:346-353.

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Full Length Research Paper

Assessment of chemical compositions of three antimalarial plants from Akure, Southwestern Nigeria: A preliminary study

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Malaria has been a menace to the health conditions of both rural and urban populations in Nigeria. Ethnobotanical survey revealed the use of *Anthocleista djalonensis* A. Chev, *Lophira alata* Banks ex C.F. Gaertn. and *Olax subscorpioidea* Oliv. in the treatment of malaria in Akure, Southwestern Nigeria. The powdered plant samples were screened for phytochemical constituents, proximate composition and mineral elements according to standard protocols. There was no significant difference in the alkaloids, cardiac glycosides, saponins and tannins of the three samples. Anthraquinones and flavonoids were altogether absent. Available carbohydrate was highest in *A. djalonensis* (66.48%) and least in *O. subscorpioidea* (66.40%) whereas crude fibre was highest in *O. subscorpioidea* (19.93%) and least in *L. alata* (15.84%). The crude protein in *A. djalonensis* and *L. alata* almost tied with the least recorded for *O. subscorpioidea* (2.39%). The fat content in the three samples was generally low. Calcium was highest in *L. alata* (11767.83±29.17 mg/kg) and least in *A. djalonensis* (7413.67±17.16 mg/kg); whereas, magnesium was highest in *A. djalonensis* (1582.33±26.10 mg/kg) and least in *O. subscorpioidea* (1180.33±33.38 mg/kg). However, *L. alata* was found to contain 333.63±1.74 mg/kg of iron while *A. djalonensis* and *O. subscorpioidea* had 301.33±4.04 and 249.68±4.72 mg/kg, respectively. Similarly, phosphorus content was highest in *L. alata* (934.58±0.51 mg/kg) and least in *O. subscorpioidea* (552.95±2.38 mg/kg). The zinc content was highest in *A. djalonensis* (80.67±2.08 mg/kg). Manganese was found to be 67.71±4.19 mg/kg in *L. alata*, 50.71±1.58 mg/kg in *A. djalonensis*, and 30.94±2.13 mg/kg in *O. subscorpioidea*. Lead tested negative in all the three samples. The plant samples contained major mineral elements and nutritive compounds. They may help to prevent opportunistic infections associated with malaria, as well as help to manage metabolic diseases. Anti-nutritive compounds and heavy metal composition in the samples are negligible and as such make the plants safe for consumption.

Key words: Malaria, *Anthocleista djalonensis, Lophira alata*, *Olax subscorpioidea,* phytochemicals, minerals, Akure, Nigeria.

INTRODUCTION

Malaria, an infectious disease caused by *Plasmodium* species, has been a menace to the health conditions of both rural and urban populations in Nigeria (NGA, 2005). Although it is a global epidemic, the incidence and severity are higher in the tropics especially in the sub-Saharan Africa, where pregnant women and children are the most susceptible (Nmorsi et al., 2007; Nguta et al., 2010). Worldwide, malaria afflicts about 40% amounting to over 300 million people annually (WHO, 2000) affecting more than 100 countries in virtually all the continents of the world (Rowe, 2006). In Nigeria, reports indicated that pregnant women are most vulnerable and hence malaria causes 10% of all deaths in pregnant women (Ayoola et al., 2008). The cost of management or eradication of malaria with conventional approach and its attendant effect on the standard of living and the economy of a nation calls for an urgent review of natural products to combat the ancient scourge.

Admittedly, plants have been used in prehistoric times and are considered effective in the management of malaria. However, and more recently, the plant world has been revisited in the ongoing fight against the disease primarily because of the deficiencies of western drugs in terms of cost, access, and drug-resistance by the malaria parasite, or more importantly the diversity of plant life, relative cheapness, acclaimed potency, and cultural relevance.

Anthocleista djalonensis A. Chev – Gentianaceae is a large tree which grows up to 20 feet; bole up to 4 cm in diameter, stilt-rooted, twig sometimes erect, spines above the leaf axils and with white flowers that are scented (Jensen and Schripsema, 2002). Traditionally, the plant is used to treat wound, malaria, constipation, dysentery, diarrhoea, hepatitis, skin infection, and inflammation (Okoli and Iroegbu, 2004; Aiyeloja and Bello, 2006).

Lophira alata Banks ex C.F. Gaertn. – Ochnaceae is usually straight, without buttress roots, but sometimes with a swollen base, and is usually clear of branches up to about 30 m with glabrous twigs and found in the subtropical and tropical moist lowland forests of Cameroun, the republic of Congo, Ivory Coast, Equitorial Guinea, Gabon, Ghana and Nigeria (Burkill, 1985). Traditionally, the bark is used in treatment of inflammation, toothache and as analgesic. In South-western Nigeria, Kayode (2006) reported the use of the leaves, stem bark, root, and seed in the treatment of malaria.

Olax subscorpioidea Oliv. – Olacaceae is a tree or sometimes a many-stemmed shrub up to 10m high of deciduous forest (Burkill, 1985). The leaf, bark, and root are used in the treatment of venereal diseases, arthritis, and rheumatism and as febrifuge (Oni, 2010).

This study was carried out to investigate the chemical constituents of three antimalarial plants used in Akure, South-western Nigeria with a view to evaluating the nutritive potentials of the plants.

MATERIALS AND METHODS

Study area

Akure is a popular metropolis in Ondo State. Akure South Local Government supports a population of over 400,000 people (NBS, 2006). The mean annual rainfall is about 1350 mm with bimodal distribution spanning between March and November; the relative humidity averaged 80% with temperature range between 23 and 30°C which is suitable for agricultural production (Folayan, 2013). Civil servants are the major inhabitants of the city which is the centre of administration of the Ondo State Government. However, farming and trading are other occupation of the residents who majored in food crops and livestock production (Folayan, 2013).

Collection and identification of plant materials

Fresh stem barks of *Anthocliesta djalonensis*, *Lophira alata* and root of *Olax subscorpioidea* were collected, and identified at the University of Ibadan Herbarium (UIH) and thereafter air-dried.

Preparation of plant samples

The dried plant samples were pulverized to coarse powder using a laboratory mill (Model 4 Arthur Thomas, USA).

Chemical analysis

The powdered plant samples were screened for phytochemical constituents, proximate composition and mineral elements according to standard protocols reported by Walsh (1971), Harbone (1973), AOAC (1990), Evans (2002), and Sofowora (2008).

Alkaloids

The powdered plant sample (500 mg) was weighed and extracted with 10 ml of hydrochloric acid (HCl). The HCl extract was then filtered with Whatman filter paper (No. 1). The filtrate of about 2.5 ml was treated with few drops of Dragendoff's reagent. A precipitate indicated the presence of alkaloids.

Anthraquinones

The powdered plant sample (500 mg) was shaken with 10 ml of benzene. The solution was filtered and 5 ml of 10% ammonium hydroxide (NH_4OH) solution was added to the filtrate. A violet colour was observed in the lower phase. It indicated presence of anthraquinones.

Cardiac glycosides

One gram (1 g) of sample was extracted with 40 ml of distilled water; the extract was placed in the oven at 100°C for 15 min. 1 ml of the preparation was added to 5ml distilled water and 2 ml Glacial Acetic Acid, and a drop of $FeCl_3$. Thereafter, 1ml of concentrated H_2SO_4 was introduced from the side of the test tube. A brown ring (with violet or green ring) signifies the presence of cardiac glycosides.

Flavonoids

A few drops of concentrated hydrochloric acid (HCl) were added to

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a small amount of an extract (0.5 g) of the plant material; development of red colour was taken as an indication of the presence of flavonoids.

Saponins

The sample (200 mg) was shaken with 5ml of distilled water and then heated to boil. Persistent frothing showed the presence of saponins.

Tannins

The sample (500 mg) was mixed with 10ml of distilled water and heated on a water bath. The mixture was filtered and ferric chloride ($FeCl_3$) was added to the filtrate. Appearance of blue black colouration showed the presence of tannins.

Polyphenols

One gram (1 g) of sample was added to 25 ml of water. The preparation was put in oven at 100°C for 15 min. The presence or absence of polyphenols was determined by adding a few drops of 1% (w/v) solution of ferric chloride followed by 1% (w/v) gelatin in sodium chloride of the same concentration. The formation of a precipitate indicated the presence of polyphenols.

Proximate composition - carbohydrate

Carbohydrate content was estimated by difference using the formula: % Available carbohydrate = 100 – (% protein + % moisture + % ash + % fibre + % fat)

Crude fibre

Two grams (2 g) of each sample was digested with 20% H_2SO_4 and NaOH solutions.

Crude protein

Half a gram (0.5 g) of each sample was weighed into a filter paper and put into a Kjeldahl flask; 10 cm^3 of concentrated H_2SO_4 was added and then digested in a fume cupboard until the solution became colourless. Distillation was carried out with 10 cm^3 of 40% NaOH. The distillate was received with 5 cm^3 of 4% boric acid in a mixed indicator till the boric acid solution turned green. Titration was done in the receiver flask with 0.01 M HCl until the solution turned red.

Fat (ether extract)

Two grams (2 g) of each sample was extracted with petroleum ether for 5 h in a Soxlet extractor.

Moisture content

Two grams (2 g) of each sample was put into the crucible and dried in an oven at 105°C overnight. The dried samples were cooled in a dessicator for 30 min and weighed to a constant weight. The percentage loss in weight was taken as the moisture content.

Total ash

Two grams (2 g) of each sample was placed in a crucible and ashed at 600°C for 3 h. The hot crucibles were cooled in a dessicator and weighed. The percentage residual weight was taken for ash content.

Mineral analysis

After wet digestion, sodium (Na), potassium (K), calcium (Ca), magnesium (Mg), copper (Cu), zinc (Zn), iron (Fe), manganese (Mn), and lead (Pb) were analyzed using Atomic Absorption Spectrophotometer (FC 210/211 VGP Bauusch Scientific AAS); phosphorus was determined using Vanadomolybdate (Yellow method). Percentage transmittance was determined at 400 nm using Spectronic 20 (Bausch and Lomb) Colorimeter.

Data analysis

Data were statistically analysed and expressed as mean ± SD. Differences in means were assessed for significance by Duncan's Multiple Range Test (DMRT) at $p<0.05$.

RESULTS AND DISCUSSION

Ethnobotanical investigation revealed the use of a recipe comprising the stem barks of *Anthocleista djalonensis, Lophira alata* and the root of *Olax subscorpioidea* in the treatment of malaria in Akure, southwestern Nigeria. Table 1 shows the plant profile of the medicinal plants. Alkaloids, anthraquinones, cardiac glycosides, flavonoids, saponins, tannins and polyphenols are the anti-nutritional factors assessed, and are in fairly low concentrations (Table 2). There was no significant difference in the alkaloids of the three samples. The amount of cardiac glycosides, saponins tannins and polyphenols were very close. Anthraquinones and flavonoids were altogether absent. Ayandele and Adebiyi (2007) reported the presence of tannins, glycosides, and saponins in the water extract of the stem of *O. subscorpioidea;* the absence of flavonoids was in line with the result obtained in this study. However, the authors reported flavonoids in the ethanol extract. This could be due to the extraction solvent as this is important for the determination of complete dissolution of bioactive compounds and the improvement of the kinetics of metabolites (Kratchnova et al., 2010). Phytochemical screening of the methanol, petroleum ether and hot-water leaf extracts of *A. djalonensis* showed the presence of tannins, saponins, flavonoids, steroids, terpenoids and cardiac glycosides (Akinyemi and Ogundare, 2014). Furthermore, Onocha et al. (2003) reported the isolation of phtalide, xanthones, monoterpene-diol, djalonenol as well as iridoid glucoside, djalonenoside from *A. djalonensis*. The isolation of lophirosides and related groups from *L. alata* has been reported by Tih et al. (1994). Phytochemical analysis of *L. alata* by Haliru et al. (2013) indicated the presence of alkaloids, tannins, saponins, anthocyanosides and

Table 1. Specimen profile of three medicinal plants used in the management of malaria in Akure, Southwestern Nigeria.

Botanical Name	Description	Voucher
Anthocleista djalonensis A. Chev	Large tree, 20 feet, stilt-rooted, spiny, flowers white, scented, sapwood; fruits green, slash brownish, granular fruits round.	UIH 12668, UIH 12618
*Sapo; Cabbage tree		UIH 21055
Lophira alata Banks ex Gaertn f.	Big tree; used to treat inflammation, toothache and cancer	UIH 14207, UIH 15627
*Ponhan; Red ironwood		
Olax subscorpioidea Oliv.	Woody shrub with leafy branchlets, flowers whitish, fruits round and bright yellow when ripe, 12mm in diameter; and has a green calyx.	UIH 16044
*Ifon; -		

a= Voucher specimen numbers of representative taxa. Source: University of Ibadan Herbarium (UIH). *Vernacular name (Yoruba); Common name.

Table 2. Phytochemical constituents of three antimalarial plants used in Akure, Southwestern Nigeria.

Parameter	Composition (%)		
	A. djalonensis	***L. alata***	***O. subscorpioidea***
Alkaloids	$0.68^a \pm 0.01$	$0.73^a \pm 0.01$	$0.61^a \pm 0.00$
Anthraquinones	$0.68^a \pm 0.01$	$0.00^a \pm 0.00$	$0.01^a \pm 0.00$
Cardiac Glycosides	$0.21^a \pm 0.00$	$0.25^a \pm 0.00$	$0.24^a \pm 0.00$
Flavonoids	$0.00^a \pm 0.00$	$0.00^a \pm 0.00$	$0.00^a \pm 0.00$
Saponins	$0.35^a \pm 0.00$	$0.31^a \pm 0.00$	$0.27^a \pm 0.00$
Tannins	$0.03^a \pm 0.00$	$0.02^a \pm 0.00$	$0.03^a \pm 0.00$
Polyphenols	$0.10^a \pm 0.00$	$0.13^a \pm 0.00$	$0.14^a \pm 0.01$

Values are mean ± SD of triplicate determinations. Means in the same row followed by the same letter are not significantly different by Duncan's Multiple Range Test (DMRT) at $p<0.05$.

reducing compounds; cardiac glycosides and anthraquinones were absent. Although, secondary metabolites function as defence against herbivores, microbes and competing plants, and as signal compounds in pollination process (Wink, 1988), they have proved to be pharmacologically important and are used as analgesics and narcotics, central nervous system stimulant, mydriatric, miotics and antihypertensive (Evans, 2002). The phytochemicals assessed in the three antimalarial plants were present in very low concentrations; this seems to question the acclaimed therapeutic activity of the plants. However, the proximate and mineral composition can be deduced to complement the bioactivity of the plants in traditional medicine.

Table 3 shows the proximate composition of the three antimalarial plants. Available carbohydrate was highest in *A. djalonensis* (66.48%) and least in *O. subscorpioidea* (66.40%) whereas crude fibre was highest in *O. subscorpioidea* (19.93%) and least in *L. alata* (15.84%). The crude protein in *A. djalonensis* and *L. alata* was almost the same with the least recorded for *O. subscorpioidea* (2.39%). The fat content in the three samples was generally low with 1.64% in *A. djalonensis* and 1.50% in *O. subscorpioidea*. A relatively high fat content was recorded for *L. alata* (3.48%). The moisture content was lowest in *L. alata* (8.47%) and highest in *O. subscorpioidea* (9.35%). In otherwords, the dry matter (DM) was highest in *L. alata* and lowest in *O. subscorpioidea.* The highest ash content was found to be in *O. subscorpioidea* (3.37%) and the least recorded in *A. djalonensis* (2.48%). The non-nutrient compositions (anti-nutrient factors) of the seed of *O. subscorpioidea* have been reported by Otori and Mann (2014). The moisture content of the plant samples was very low; this is an indication that the plants could withstand long storage. Carbohydrate was high in the samples; carbohydrates provide the energy required for normal physiological functions; they help to power cells and tissues in the body. Crude fibre, made up of cellulose with little quantity of lignin, is indicative of the level of non-digestible

Table 3. Proximate composition of three antimalarial plants used in Akure, Southwestern Nigeria.

Parameter	Composition (%)		
	A. djalonensis	*L. alata*	*O. subscorpioidea*
Ash	$2.48^{b} \pm 0.34$	$2.57^{b} \pm 0.09$	$3.37^{a} \pm 0.14$
Carbohydrate	$66.48^{a} \pm 0.62$	$66.32^{a} \pm 0.94$	$63.40^{b} \pm 0.37$
Crude Fibre	$19.27^{a} \pm 0.27$	$15.84^{b} \pm 0.16$	$19.93^{a} \pm 0.08$
Crude Protein	$3.38^{a} \pm 0.23$	$3.31^{a} \pm 0.21$	$2.39^{b} \pm 0.12$
Fat	$1.64^{b} \pm 0.10$	$3.48^{a} \pm 0.44$	$1.50^{b} \pm 0.12$
Moisture	$8.50^{b} \pm 0.36$	$8.47^{b} \pm 0.47$	$9.35^{a} \pm 0.19$

Values are mean ± SD of triplicate determinations. Means in the same row followed by the same letter are not significantly different by Duncan's Multiple Range Test (DMRT) at p<0.05.

Table 4. Mineral element composition of three antimalarial plants used in Akure, Southwestern Nigeria.

Mineral	Composition (mg/kg)		
	A. djalonensis	*L. alata*	*O. subscorpioidea*
Calcium	$7413.67^{c} \pm 17.16$	$11767.83^{a} \pm 29.17$	$11667.67^{b} \pm 28.88$
Copper	$22.67^{a} \pm 2.08$	$8.38^{b} \pm 0.58$	$8.66^{b} \pm 0.59$
Iron	$301.33^{b} \pm 4.04$	$333.63^{a} \pm 1.74$	$249.68^{c} \pm 4.72$
Lead	$0.00^{a} \pm 0.00$	$0.00^{a} \pm 0.00$	$0.00^{a} \pm 0.00$
Magnesium	$1582.33^{a} \pm 26.10$	$1227.36^{b} \pm 7.47$	$1180.33^{c} \pm 33.38$
Manganese	$50.71^{b} \pm 1.58$	$67.71^{a} \pm 4.19$	$30.94^{c} \pm 2.13$
Phosphorus	$715.47^{b} \pm 2.75$	$934.58^{a} \pm 0.51$	$552.95^{c} \pm 2.38$
Potassium	$0.55^{a} \pm 0.03$	$0.18^{b} \pm 0.01$	$0.76^{a} \pm 0.01$
Sodium	$0.61^{a} \pm 0.04$	$0.22^{b} \pm 0.02$	$0.77^{a} \pm 0.02$
Zinc	$80.67^{a} \pm 2.08$	$57.25^{b} \pm 0.46$	$57.44^{b} \pm 1.49$

Values are mean ± SD of triplicate determinations. Means in the same row followed by the same letter are not significantly different by Duncan's Multiple Range Test (DMRT) at p<0.05.

carbohydrate (Onwuka, 2005; Akpaibo and Ikpe, 2013). Low levels are reported for the samples. This is good as high level could cause indigestion and irritation of the bowels. Lipids are good sources of energy. They act as insulators and protect delicate organs of the body, and are important in many cellular functions; this study, however, reports low values of fat in the samples. Protein and ash are generally low in the samples. Proteins are useful in bakery and confectioneries (Khalil et al., 2012) whereas ash content represents the mineral matter of food samples (Onwuka, 2005).

Calcium, magnesium, phosphorus, and iron were found to be very high in the three samples (Table 4). Calcium was highest in *L. alata* (11767.83±29.17 mg/kg) and lowest in *A. djalonensis* (7413.67±17.16 mg/kg) whereas magnesium was highest in *A. djalonensis* (1582.33±26.10 mg/kg) and least in *O. subscorpioidea* (1180.33±33.38 mg/kg). However, *L. alata* was found to contain 333.63±1.74 mg/kg of iron while *A. djalonensis* and *O. subscorpioidea* had 301.33±4.04 mg.kg and 249.68±4.72mg/kg respectively. Similarly, phosphorus content was highest in *L. alata* (934.58±0.51 mg/kg) and least in *O. subscorpioidea* (552.95±2.38 mg/kg). Potassium and sodium were found to be very low in the plant samples with highest values recorded in *O. subscorpioidea* and lowest values in *A. djalonensis* in each case. The zinc content obtained for *A. djalonensis* was 80.67±2.08 mg/kg whereas *L. alata* and *O. subscorpioidea* almost tied in their zinc content with values 57.25±0.46 and 57.44±1.49 mg/kg, respectively. Manganese was found to be 67.71±4.19 mg/kg in *L. alata*, 50.71±1.58 mg/kg in *A. djalonensis*, and 30.94±2.13 mg/kg in *O. subscorpioidea*. The amount of copper present in *A. djalonensis* was 28.67±2.08 mg/kg, 8.66±0.59 in *O. subscorpioidea* and 8.38±0.58 in *L. alata.* Lead tested negative in all the three samples screened. Mineral elements have been considered to be of great importance in the prevention of disease and in the general well-being of individuals (Nielsen, 2000) as they fulfil a critical function in physiological and biochemical processes. Moreover, these elements are our natural resources and are sourced from both plants and animals. The major elements are necessary for life processes; calcium and magnesium, for example, are essential for teeth and bone formation, for healthy blood vessels, energy formation, and in the transmission of nerve impulses (Griffith, 1988; Soetan et al., 2010). Potassium and sodium are needed in the maintenance of body fluid, blood pressure, muscle contraction, nerve transmission, and metabolism (Anon., 2014). Phosphorus plays a key role in the absorption of carbohydrates, proteins, and fat and in the biochemical reactions in the body. Iron is important in haemoglobin formation and in the production of energy. In the regulation of immune system, iron and zinc are essential. Copper is involved in respiratory and red blood cell function, whereas zinc helps to maintain taste and sensitivity (Mayer and Goldberg, 1990; Saraf and Samant, 2013). Manganese features in enzyme activities, glucose, protein and fat metabolism and in the maintenance of healthy immune system (Anon., 2014).

Conclusion

This study has provided an assessment of the chemical composition of three antimalarial plants. *A. djalonensis, L. alata* and *O. subscorpioidea* contained major mineral elements and nutritive compounds. These substances will serve well as food supplements. They may help to prevent opportunistic infections associated with malaria, as well as help to manage metabolic diseases. Anti-nutritive compounds and heavy metal composition are negligible and as such make the plants safe for consumption.

Conflict of interest

The authors declare no conflict of interest.

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REFERENCES

Aiyeloja AA, Bello OA (2006). Ethnobotanical potentials of common herbs in Nigeria: A Case study of Enugu State. Educ. Res. Rev. 1(1):16-22.

Akinyemi AI, Ogundare AO (2014). Antibacterial properties of the leaf extracts of *Anthocleista djalonensis* A.Chev on some pathogenic organisms. Eur. J. Med. Plants, 491:75-85.

Akpaibo UD, Ikpe EE (2013). Proximate composition and nutrient analysis of *Aneilema aequinoctiale* leaves. Asian J. Plant Sci. Res. 3(2):55-61.

Anon (2014). Elements comprising the human body: the roles of minerals and elements in life processes. www.mii.org/periodic/LifeElement.html. Accessed: 26th August, 2014.

AOAC (1990). Official Methods of Analysis. Association of Analytical Chemists. 15th Edition. Washington, DC, USA. pp. 112-135.

Ayandele AA, Adebiyi AO (2007). The phytochemical analysis and antimicrobial screening of extracts of *Olax subscorpioidea*. Afr. J. Biotech. 6(7):868-870.

Ayoola GA, Coker HAB, Adesegun SA, Adepoju-Bello AA, Obaweya K, Ezennia, Atangbayila TO (2008). Phytochemical screening and antioxidant activities of some selected medicinal plants used for malaria therapy in Southwestern Nigeria. Trop. J. Pharm. Res. 7(3):1019-1024.

Burkill HM (1985). The Useful Plants of West Tropical Africa. Royal Botanic Gardens, Kew.

Evans WC (2002). Trease and Evans Pharmacognosy. 15th Edition. W.B. Saunders, London. pp. 214-393.

Folayan JA (2013). Socio-economic analysis of Fadama farmers in Akure South Local Government Area of Ondo State, Nigeria. Am. J. Hum. Soc. Sci. 1(1):10-17.

Griffith HW (1988). Complete guide to vitamins, minerals, and supplements. Fisher Books, Arizona.

Haliru M, Akande TA, Ayilara AT, Mustapha AT (2013). Phytochemical analysis and antibacterial activity of *Khaya grandifoliola* and *Lophira alata* against some selected clinical bacterial isolates. Wudpecker J. Med. Plants. 2(1):7-15.

Harbone JB (1973). Phytochemical Methods. Chapman and Hall Ltd., London. pp. 49-188.

Jensen SR, Schripsema J (2002). Chemotaxonomy and pharmacology of Gentianaceae. In: Struwe, L. and Albert, V. (eds). Gentianaceae - Systematics and Natural History. Cambridge University Press, United Kingdom. pp. 573-631.

Kayode J (2006). Conservation of indigenous medicinal botanicals in Ekiti State, Nigeria. J. Zhejiang Univ. Sci. B7:713-718.

Khalil E, Esoh R, Rababah T, Almajwal AM, Ahidatt MH (2012). Minerals, proximate composition and their correlations of medicinal plants from Jordan. J. Med. Plants Res. 6(47):5757-5762.

Kratchnova M, Denev P, Ciz M, Lojek A, Mihailov A (2010). Evaluation of antioxidant activity of medicinal plants containing polyphenol compounds. Comparison of two extraction extraction systems. Acta Biochimica Polonica 57:229-234.

Mayer J, Goldberg J (1990). Dr. Jean Mayer's diet and nutrition guide. Pharos Books, New York.

NBS (2006). National Bureau of Statistics, National Population Commission, Abuja, Nigeria.

Nguta JM, Mbaria JM, Gakuya DW, Gathumbi PK, Kiama SG (2010). Antimalarial herbal remedies of Msambweni, Kenya. J. Ethnopharmacol. 128:424-432.

Nielsen FH (2000). Importance of making dietary recommendations for elements designated as nutritionally beneficial, pharmacologically beneficial, or conditionally essential. The J. Tr. Elmts Exp. Med. 13: 113-129.

Nigeria Government in Action (NGA) (2005). Report from Presidential Research and Communication Unit, Office of Public Communication, Abuja.

Nmorsi OPG, Ukwandu NCD, Egwunyenga AO (2007). Antioxidant status of Nigerian children with *Plasmodium falciparum* malaria. Afr. J. Microb. Res. pp. 61-64.

Okoli AS, Iroegbu CU (2004). Evaluation of extracts of *Anthocleista djalonensis*, *Nauclea latifolia and Uvaria afzalii* for activity against bacterial isolates from cases of non-gonococcal urethritis. J. Ethnopharmacol. 92(1):135-144.

Oni SO (2010). *In vitro* study of the effects of aqueous extracts of *Olax subscorpioidea* and *Lonchocarpus cyanescens* on monoamide oxidase activity from rat brain. Unpublished B.Sc. dissertation, Federal University of Agriculture, Abeokuta, Nigeria.

Onocha PA, Okorie DA, Connolly JD, Krebs HC, Meier B, Habermehl GG (2003). Cytotoxic activity of the constituents of *Anthocleista djalonensis* and their derivatives. Nig. J. Nat. Prod. Med. 7:58-60.

Onwuka GI (2005). Food analysis and instrumentation: theory and practice. Naphtalic Prints, Surulere, Lagos, Nigeria pp. 63-98; 219-230.

Otori AA, Mann A (2014). Determination of chemical composition, minerals and antinutritional factors of two wild seeds from Nupeland, North Central Nigeria. Am. J. Chem. Appl. 1(1):20-26.

Rowe AK (2006). The burden of malaria among African children in the year 2000. Intl. J. Epidemiol. 35:691-704.

Saraf A, Samant A (2013). Evaluation of some minerals and trace elements

in *Achyranthes aspera* Linn. Intl. J. Pharm. Sci. 3(3):229-233.
Soetan KO, Olaita CO, Oyewole OE (2010). The importance of mineral elements for humans, domestic animals and plants: A review. Afr. J. Food Sci. 4(5):200-222.
Sofowora A (2008). Medicinal plants and traditional medicine in Africa. 3rd Edition. Spectrum Books Ltd., Ibadan, Nigeria. pp. 199-204.
Tih EA, Tih RG, Sondengam BL, Martin MT, Bodo B (1994). Minor bioflavonoids from *Lophira alata*. J. Nat. Prod. 57:971.
Walsh LM (1971). Instrumental methods for analysis of soils and plant tissue. Soil Science Society of America Inc. Madison Wisconsin, USA.
Wink M (1988). Plant breeding: importance of secondary metabolites for protection against pathogen and herbivores. Theor. Appl. Gen. 75:225-233.
World Health Organization (WHO) (2000). Severe *Falciparum* malaria. Trans. Royal Soc. Trop. Med. Hyg. 94(Suppl. 1):551-590.

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Review

Vital role of herbal medicines in women's health: A perspective review

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This is a perspective review of medicinal plants useful traditionally for women's healthcare in countries like India. Medicinal plants have a significant role in women's healthcare in many rural areas. Plants with therapeutic efficacious observations have historically been used as a starting point in the development of new drugs, and modern pharmaceuticals have been derived from them. A review about the therapeutic effectiveness, safety and best use of herbals in day to day practice to get rid of many diseases adds value to making use of herbals in this context. Kitchen remedies are the easy access for women for their cost effective health care. Many of the dietary health practices by women reflect their health consciousness. Herbal remedies include medicinal herbs and ayurveda herbal remedies for common disorders among women such as urinary tract infection, pubertal changes, post-menopausal syndrome, hot flushes, menopause, poly cystic ovarian syndrome, bacterial vaginosis, yeast infections, infertility, delayed labor, low breast milk production, abortion and other female disorders.

Key words: Medicinal plants, menopausal syndrome, herbal remedies, infertility.

INTRODUCTION

Women play an important role in the society as well as in the total life scenario on earth. Despite obvious differences between women and men biologically, psychologically, and socially, the act of differentiating women's health from that of men arose in Western medicine only in the last two decades of the twentieth century. Only health care providers who are specialized in areas related to reproduction were expected to be knowledgeable about issues particular to women. Women from rural sector or modern society rely on herbals for their health care and beauty care (Beal, 1998). Herbal remedies for women include medicinal herbs and Ayurveda herbal remedies for problems like urinary tract infection, pubertal changes, post-menopausal syndrome, hot flushes, menopause, poly cystic ovarian syndrome, bacterial vaginosis, yeast infections, infertility, delayed labor, low breast milk production, abortion and other female disorders. Women have handed down information from mother to daughter on how herbs can remedy some of the common maladies of life. Women, like the moon, change in cycles.

Through menarche (the first menses), menstrual cycles (Bourdy and Walter, 1992), pregnancy, nursing, and menopause, herbs have been a common denominator for

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the wise woman and those she comforts. Medicinal plants are sometimes referred to as being phytoestrogenic or phytoprogesteronic. This is because some plants have molecular structures similar to the hormones estrogen (phytoestrogenic) and progesterone (phytoprogesteronic). They can occupy the receptor sites in the body that would normally be taken up by these hormones. Herbs are beautiful allies for women to use throughout their lives. Medicinal plants, because of their high values and least side effects, are used by women around the globe. The practice is increasing and seems quite encouraging, as it reverts back to the olden days where herbs and its usage found interesting episodes.

The cost and availability of herbs and their utilization resources transferred from one generation to another keep the information alive and useful to all. Since women play a multirole and face a variety of problems, special health care is needed. Medical care is becoming costly and much painful; its affordability is no within the reach of the poor. So there is a great demand for usage of medicinal among women in both rural and urban sectors. They are nourishing, comforting, and have stood the test of time for millions of women over thousands of years (Overk et al., 2008).

WOMEN HEALTH CARE

World scenario (World Health Organization, 2008)

Around the globe, women at all ages suffer from variety of diseases and health issues. Stress and poor health care, malnutrition and many aspects worsen their health. Medicinal plants have role in taking care of such issues. In order to understand the modern definition of women's health, it is important to understand women's health care viewed by the medical and medical research establishments. Traditionally, the health of women has been seen as synonymous with maternal or reproductive health. Clearly, the Western medical profession's view of women's health as maternal was concordant with societal mores that valued women mainly for their ability to bear children. Childbirth and sexually transmitted diseases, and cervical cancer have been the most important health issues for women in all ages and places.

Indian context (Subramanian et al., 2006)

Due to the unpleasant risks and side effects of long-term pharmaceutical treatment for women's health conditions, specifically menstruation and menopause, women's healthcare and the search for alternative treatment options have become an important focus of global scientific research. Women are getting more stress, and lack of self-care and poor nourishment lead to anemia and other malnutrition symptoms. Women’s ability to tolerate suffering and their reluctance to be examined by male personnel are additional constraints in their getting adequate health care in the Indian scenario. Pollution and industrial wastes badly reflect on metabolism and lead to health problems in women. In addition, the smoke from household biomass has serious impact, such as eye problems, respiratory problems, chronic bronchitis and lung cancer among women, as the exposure time is more in our social setup. It may lead to anemia in those women susceptible to carbon monoxide toxicity. Mortality, smoking, chewing tobacco and alcohol use were four separate binary outcomes in the analysis in Indian scenario.

Smoking, drinking alcohol, and chewing tobacco also show graded associations with socioeconomic status within indigenous groups. Socioeconomic status difference substantially accounts for the health inequalities between indigenous and non-indigenous groups in India. However, a strong socioeconomic gradient in health is also evident within indigenous populations, reiterating the overall importance of socioeconomic status for reducing population-level health disparities, regardless of indignity.

Women healthcare in India

Women’s health can be seriously affected by many factors such as stress, emotional, physical and so on problems. The major problems are menstrual irregularities, mental health, and malnutrition status like anaemia. The effective intake of herbals may lead to better results in this regard. Women from occupational sector suffer from stress, reluctance about their food habits, more prone to ill effects in their health. As women shoulder all responsibilities in their homes and society, adequate care must be provided to handle their problems (Sethuraman et al., 2006).

The practice of traditional medicine is widespread in China, India, Japan, Pakistan, Sri Lanka and Thailand. In China, about 40% of the total medicinal consumption is attributed to traditional tribal medicines. In Japan, herbal medicinal preparations are more in demand than mainstream pharmaceutical products (Lim, 1993).

The modern field of women's health includes the study of illnesses and conditions that are unique to women, more common or serious in women, have distinct causes or manifestations in women, or have different outcomes or treatments in women. Since the 1980s, research on gender differences in health and disease has had important implications for the treatment and prevention of a variety of common serious illnesses, including heart disease, stroke, lung cancer, depression, colon cancer, and dementia. Research in all these areas is ongoing. A greater understanding of the factors influencing women's health from a biological perspective has been paralleled by a greater understanding of the psychosocial and societal factors that affect women's health status. Differences in employment patterns also result in fewer women being medically insured than men, strongly affecting access to health care and health status.

The field of women's health seeks to promote an understanding of the biological and psychosocial factor affecting women's health, and to integrate this understanding into public health initiatives, including training of health care providers. Recognition by the medical research establishment of the need to study health and disease in women as well as men has been essential to this new paradigm. Despite the strong influence of biological factors, psychosocial issues still remain the single most important determinant of health status for many women.

The importance of herbals in traditional healthcare practices, providing clues to new areas of research and in biodiversity conservation is now well recognized. However, information on the uses of plants for medicine is lacking in many interior areas. Developmental activities and changing socio-economic conditions have implication on traditional knowledge.

ROLE OF HERBALS IN WOMEN'S HEALTH CARE

Among the women population, a large percentage suffer from anemia and related issues. Women have unique health concerns, and Botanic Choice has natural solutions. Nature's medicine chest provides the support you need for strong bones, bladder health, hormone balance and heart health.

Gastro-intestinal ailments

Although women may experience heartburn, they generally have less damage in their esophagus than men. Because women are more sensitive to irritants, they may experience heartburn more strongly than men. The common gastric disorders of women are: nausea, gastritis, gallstones, irritable bowel syndrome (IBS), and colonic disorders.

Gynaecological problems (Pinn, 2001)

Gynaecological problems are abnormal uterine bleeding and endometrial ablation. Endometrial ablation is a form of minimally invasive procedure in the treatment of heavy menstrual bleeding. Endometriosis is a gynaecological problem. It is affects women usually between the ages of 30 - 45 years old. Many problems produce endometriosis such as heavy and sometimes irregular periods and also gynaecological problems.

Uterine fibroids are muscle swellings that are found in the womb. Fibroids are very common in women. They are commonly found in women between the ages of 35 - 45 years old.

Osteoporosis is the commonest long term complication of the menopause. Severe cases of osteoporosis can result in spinal problems and a decrease in height or in hip fractures with minimal trauma. The instance of coronary heart disease and strokes is much lower in women before the menopause age. Wild yam root (*Dioscorea villosa*) is a member of the Dioscoreaceae family.

Wild yam improves liver and kidney function and can lessen dysmenorrhea and ovarian pain. It is anti-inflammatory, antispasmodic, diuretic, and nutritive, and a cholagogue (improves liver function). Wild yam contains diosgenin, which is a precursor to progesterone and was once used to make birth control pills. Today, wild yam, valued as an herb, is useful for dysmenorrhea, infertility, menopause, menstrual cramps, ovarian pain, and threatened miscarriage. Soy products, which are eaten widely in the Far East, are hypothesized to play a role in this region's: lower rates of cancers and heart disease and menopausal symptoms. Soy products such as beans, tempeh, tofu, soy milk, and miso contain isoflavones, an antioxidant which can reduce hot flashes and help inhibit tumor growth and cancer.

Plants, namely *Tinospora cordifolia,* whose leaves are used as health tonic; *Delonix regia*, whose flowers are used for treating dysmenorrhoea; Buteamonosperma whose bark are used for menorrhagia and leucorrhoea are found similar to those published in the literature (Vidyasagar and Prashantkumar, 2007). Further scientific assessment of these medicines on phytochemistry, biological activity and clinical studies is however greatly needed. Most commonly useful herbs are: *Andrographis paniculata Nees, Abrus precatorius L., Butea monosperma Roxb., Caesalpinia bonducella L. , Catharanthus roseus L., Celosia argentia L., Crotalaria prostrate Rottl., Lawsonia innermis L., Maytenus senegalensis Lam., Mimosudica L., Striga densiflora Benth., Tinospora cordifolia Willd* and *Tridax procumbens*

Menopause is the time of life when a women stops having periods (Brian, 2009). The periods stop because the ovaries stop producing the normal amounts of oestrogen and progesterone hormones. Hot flushes and night sweats are very common during menopause. *Cinnamomum verum, Pueraria lobata, Ruta graveolens, Glycine max, Dioscora villosa, Cimicifuga racemosa, Vitex agnus, Angelica sinensis, Oenothera biennis, Ginkgo biloba, Trifolium pretense, Agrimonia pilosa hedeb, Ailanthus altissima (Mill) swingle* are commonly employed in the treatment of gynaecological conditions.

Infertility or its concern worries a larger number of couples. It has been shown that if a couple is having regular unprotected sexual intercourse, then there is 80% chance of conception after 12 months and 90% chance of conception after 18 months.

There are three main causes of infertility. A woman needs to produce eggs regularly and at the right time of her menstrual cycle, the man needs to produce sperm of the right quality and quantity, and the two need to be able to meet and therefore the women’s fallopian tubes need to be open and undamaged. About 25% of infertility is due to lack of eggs; about 25% is due to a problem with the sperm, about 25% is due to tubal problems and for the balance 25%, the reason for infertility is not known.

Numerous studies have documented that health care consumers all over the world are spending money for alternative therapies and that billions of dollars are spent in the United States alone. Women use conventional health care services more frequently than men; thus, it is not surprising that women account for approximately two thirds of health care appointments for complementary and alternative therapies. The traditional conceptual frameworks of herbal medicine, homeopathy, acupuncture, and acupressure are presented, and common clinical applications to women's reproductive care are discussed**.**

Ethnobotanical data collection to select pharmacologically active species was carried out within a clearly defined therapeutic context: those plants used during the course of a woman's reproductive life. Various concepts, behaviours and practices relating to menstruation, pregnancy, birth and birth control were examined in detail from an ethno pharmacological point of view. A list of selected species of particular interest is proposed for further study.

Symptoms associated with menopause can greatly affect the quality of life for women. Botanical dietary supplements have been viewed by the public as safe and effective despite a lack of evidence. Taken together, these data indicate a need to reprioritize the order in which the bioassays are performed for maximal efficiency of programs involving bioassay-guided fractionation. In addition, there are possible explanations for the conflicts in the literature over the estrogenicity of *Cimicifuga racemosa* (Black cohosh).

Despite widespread use, there has been surprisingly little research on the outcomes or the potential risks of using herbal therapies during pregnancy. Similarly, phytoestrogens have become one of the fashionable areas of herbal treatment, although with remarkably little evidence of benefit. Studies have been carried out to assess their effectiveness in cardiovascular disease and osteoporosis but not their effect on irregularities of menstruation. Current herbal treatment in this area comes from traditional use, laboratory work; and a lack of adequate clinical trials make it impossible to suggest which remedies may be of benefit.

Estrogen replacement therapy is one of the most commonly prescribed medicines in the United States by traditional medical professionals (Russell, 2002). Over the past decade, the market for complementary/ alternative therapies for hormone replacement has dramatically increased. Women are seeking more "natural" alternatives to treat menopausal symptoms. Several popular herbal therapies for menopausal symptoms include phytoestrogens, black cohosh (*Cimicifuga racemosa*), dong quai (*Angelica sinensis*), chast tree (*Vitex agnus-castus*), and wild Mexican yam (Tiran, 2006).

Women approaching menopause frequently resort to complementary therapies and natural remedies, especially herbal medicines. Nurses working with mature women, both in communities and hospitals, debate about these remedies, yet are unable to answer women's questions, or know where to get information. However, with the increased use of complementary therapies generally, it is imperative that nurses recognize the parameters of their personal practice and appreciate the possible problems which may arise from ill-informed use of natural remedies, such as herb-drug interactions. This article provides an overview of herbal remedies popularly self-administered by women in their peri-menopausal period.

The effects and safety of several remedies are explored to facilitate nurses to offer accurate, comprehensive and evidence-based information to patients. The issue of integration of herbal medicine into mainstream management of menopausal symptoms is also debated as a means of providing optimum and safe care to women at this time.

General tonic

Medicinal plants used as general tonic include: *Medicago sativa, Andrographis paniculata, Chicorium intybus,, Morinda citrifolia, Olea europea, Panax quinquefoilus, Zingiber officinale.* Moderate malnutrition continues to affect 46% of children under five years of age and 47% of rural women in India. Women's lack of empowerment is believed to be an important factor in the persistent prevalence of malnutrition.

In India, women's empowerment often varies by community, with tribes sometimes being the most progressive. In addition to the known investments needed to reduce malnutrition, improving women's nutrition, promoting gender equality, empowering women, and ending violence against women could further reduce the prevalence of malnutrition in this segment of the Indian population (Uniyal et al., 2006).

Headache

Plants useful in headache include (El-Mallakh et al., 1991b): *Tumera diffuse, Ilex paraurensis.* A number of clinical reports have revealed an association between the use of alcohol and drugs and the onset or exacerbation of headaches. The following characteristics were noted in the 236 respondents: 1) Over 89% reported having experienced some type of headache; 2) headache-free individuals were significantly older than headache sufferers; 3) women were much more likely to have migraine headaches than men; 4) Onset of migraines occurred prior to onset of substance use, while onset of tension headaches occurred after onset of substance use. Although associational data must be interpreted with caution, an intriguing hypothesis compatible with the finding is that migraines may play a role in the genesis of substance use, while substance use may play a role in the genesis of tension headaches.

Fever, cough and cold

Medicinal plants are the inexpensive drugs for all categories of people in the world because of their less serious side effects compared to the synthetic ones. Plants useful in fever, cough and cold include *Arnica Montana, Berberis vulgaris, Bupleurum falcatum, Eucalyptus globules, Hibiscus sabdariffa, Hyssopus officinalis, Datura stromonium, Althea officinalis, Urginia maritime, Rumex crispus, Eriodictyon californicum, Astragalus membarneceous, Trogonellafoenum- graecum, Tanacetum parthenium, Glycyrriza glabra, Verbascum densiflorum, Agastache rugosa gynae,* and *Baphicacanthus cusia (Nees) Bremek.*

Cancer (Desai et al., 2008)

About one out of every 4-60 women will develop ovarian cancer in their lifetime. It has been noted that the more children a woman has, the lower her risk for ovarian cancer. Breast cancer is the most common form of cancer in women. It is the major cause of death from cancer for women aged between 30 and 60 years. Men can also suffer from breast cancer but compared with women it is a rare occurrence with an incidence rate of about 1% compared with the rate in women. Cervical cancer is one of the most common cancers affecting women. It occurs in the cervix which is the lower part of the womb protruding into the vagina. Cervical cancer is divided into two stages; early or pre-invasive stage, and the late or invasive stage. Women who have had several pregnancies or several sexual partners seem more at risk to cervical cancer. There are indications that cervical cancer may be caused by a virus, the wart virus.

Medicinal plants useful in cancer

Medicinal plants useful in cancer include *Momordica charantia, Tricosanthes kirilowii,Codonopsis pilosula,Vitis vinifera, Camelia sinensis, Lavendula angustifolia, Podophyllum peltatum, Viscus álbum; Pinus pinaster, Rosmarinus officinalis, Ganoderma lucidum, Scutellaria species, Glycinemax, Thuja occidentalis, Withania somnifera, Allium sativum, Panax ginseng.*

Since 1986, over 40,000 plant samples have been screened, but thus far only five chemicals showing significant activity against AIDS have been isolated. Three are currently in preclinical development. Before being considered for clinical trials in humans, these agents must show tolerable levels of toxicity in several animal models. For AIDS, three agents are presently in preclinical or early clinical development.

OCCUPATIONAL HEALTH PROBLEMS OF WOMEN; HERBAL REMEDY

Occupational health problems occur due to work or because of the kind of work you do. These problems can include cuts, broken bones, sprains and strains, or amputations, repetitive motion disorders, hearing problems caused by exposure to noise, vision problems or even blindness, Illness caused by breathing, touching or ingesting unsafe substances, Illness caused by exposure to radiation, exposure to germs in healthcare settings. Good job safety and prevention practices can reduce your risk of these problems: try to stay fit, reduce stress, set up your work area properly, and use the right equipment and gear.

Only a small proportion of exposed workers develop occupational asthma (Kelly-Pieper, 2009). Workers most likely to develop the disease are those with a personal or family history of allergies or asthma and frequent exposure to highly sensitizing substances. But the disease also can develop in persons with no known allergies.

Occupational asthma may be suspected whenever a worker begins to develop respiratory symptoms. It may take several years to develop. A thorough physical examination and medical history for a worker with asthma symptoms should include a detailed listing of his or her work history and workplace conditions.

As women move beyond their traditional occupations, they meet new health hazards which may either replace or add to their existing occupational exposure. Women's labour force participation rates have increased steadily, and not only in the industrialized countries.

The dramatic economic successes of the newly industrialized states of Asia, for example, are substantially a reflection of increasing feminization of labor in this region. In these economies, female’s workforce participation rates increased far more rapidly than male from the 1960s, although their jobs were largely less-skilled and poorly paid. Women workers formed the largest pool of workers in export-oriented light industries, such as electronics and textiles, which underpinned economic expansion.

The major reason it is necessary to develop specific tools for research into women's occupational health problems is that the labour force is still very much divided by sex, so women and men do very different work and are exposed to different risks. In order for men and women to be evenly distributed across the job market, about three quarters of women would have to change jobs. A recent study of workers in North Carolina, U.S.A. puts this figure at 76 per cent, even higher than that for racial segregation: 55%. Despite considerable progress in integrating women into the labour force, women are still found in jobs where employment conditions are relatively unfavourable. This sexual division of labor affects women's health in at least six ways:

1) Women's jobs have specific characteristics (repetition, monotony, static effort, multiple simultaneous responsibilities) which may lead over time to changes in physical and mental health;

2) Spaces, equipment and schedules designed in relation to the average male body and lifestyle may cause problems for women;

3) Occupational segregation may result in health risks for women and men by causing task fragmentation, thereby increasing repetition and monotony;

4) Sex-based job assignment may be vaunted as protecting the health of both sexes and thus distract from more effective occupational health promotion practices;

5) Discrimination against women is stressful in and of itself and may affect mental health;

6) Part-time workers are excluded from many health-promoting benefits such as adequate sick leave and maternity leave.

Recent years have seen an increase in the number of women in the labor force, and public health practitioners, workers and scientists are starting to include women's concerns in their occupational health activities

COMMERCIAL IMPACT OF WOMEN'S HEALTH CARE PRODUCTS IN INDIAN ECONOMY

Health inequities, "the avoidable inequalities in health between groups of people within countries and between countries", are shaped by the social and economic conditions of people's lives. In 2002 the World Health Organization (WHO) released the Madrid Statement, saying: *"to achieve the highest standard of health, health policies have to recognize that women and men, owing to their biological differences and their gender roles, have different needs, obstacles and opportunities."*

Consistent with this, the government's approach to developing the National Women's Health Policy will be based on a principle of gender equity. To achieve gender equity in health, both women and men need health policies that target their specific or unique needs (Douglas, 2006).

DISCUSSION

Medicinal plants are the inexpensive drugs for all categories of people in the world because of their less serious side effects compared to the synthetic ones. The effects on health of women's multiple roles are still poorly understood. Longitudinal studies could be valuable here too in disentangling the impact of different roles and responsibilities at different stages of the life-cycle. If much of the current literature on women and paid work, especially which concerned mental health, is ambiguous or contradictory, it frequently reflects inadequate research design and an unjustifiable level of generalization about women's lives. Medicinal plants are sometimes referred to as being phytoestrogenic or phytoprogesteronic. This is because some plants have molecular structures similar to the hormones estrogen (phytoestrogenic) and progesterone (phytoprogesteronic). They can occupy the receptor sites in the body that would normally be taken up by these hormones. The occupational health of women in sex work varies with the meanings, customs and contexts of sex work in their local area.

CONCLUSION

Medicinal plants are easily available and without any unwanted side and adverse effects. Comparing the herbal medicine and allopathic, the allopathic system of medicine cost is high and the adverse and side effect is more. It is not possible to take continuously allopathic medicine over a certain period of time to remedy diseases. Although, herbal medicines have positive results of 100% without side effects. Nowadays, we can see increase in the number of women in the labour force; public health practitioners, workers and scientists are starting to include women's concerns in their occupational health activities.

Conflict of interest

The authors have not declared any conflict of interest.

REFERENCES

Beal MW (1998). Women's use of complementary and alternative therapies in reproductive health care. J. Nurse Midwifery, 43(3):224-234.

Bourdy G, Walter A (1992). Maternity and medicinal plants in Vanuatu. I. The cycle of reproduction. J. Ethnopharmacol. 37(3):179-96.

Brian JD, Frasor J, Lauren EB, Locklear TD, Perez A, Gomez- Laurito J, Mahady GB (2009). Estrogenic effects of herbal medicines from Costa Rica used for the management of menopausal symptoms Menopause. 16(4):748-755.

Desai AG, Qazi GN, Ganju RK, El-Tamer M, Singh J, Saxena AK, Bedi YS, Taneja SC, Bhat HK (2008). Medicinal plants and cancer chemoprevention. Curr. Drug Metab. 9(7):581-591.

Douglas WE (2006). How social marketing works in health care BMJ. 332(7551): 1207-1210.

El-Mallakh RS, Kranzler HR, Kamanitz JR (1991b). Headaches and psychoactive substance use. Headache, 31:584-587.

Lim Lin Lean (1993). "The Feminization of Labour in the Asia-Pacific Rim Countries: From Contributing to Economic Dynamism to Bearing the Brunt of Structural Adjustments", in Naohiro Ogawa, G.W. Jones and J.G. Williamson (eds), Human Resources in Development along the Asia-Pacific Rim (Singapore: Oxford University Press, 1993) Tables 6.2-6.3, pp. 177-181.

Kelly-Pieper K, Patil SP, Busse P, Yang N, Sampson H, Li XM, Wisnivesky JP, Kattan M (2009). Safety and tolerability of an antiasthma herbal Formula (ASHMI) in adult subjects with asthma: a randomized, double-blinded, placebo-controlled, dose-escalation phase I study. J. Altern. Complement Med. 15(7):735-43.

Overk CR, Yao P, Chen S, Deng S, Imai A, Main M, Schinkovitz A, Farnsworth NR, Pauli GF, Bolton JL (2008). High-content screening and mechanism-based evaluation of estrogenic botanical extracts. Comb. Chem. High Throughput Screen. 11:283-293.

Pinn G (2001). Herbs used in obstetrics and gynaecology. Aust. Fam. Physician, 30(4):351-354, 356.

Russell L, Hicks GS, Low AK, Shepherd JM, Brown CA (2002). Phytoestrogens: a viable option? Am. J. Med. Sci. 324(4):185-188.

Sethuraman K, Lansdown R, Sullivan K (2006). Women's empowerment and domestic violence: the role of sociocultural determinants in maternal and child under nutrition in tribal and rural communities in South India. Food Nutr. Bull. 27:128-143.

Subramanian SV, Davey Smith G, Subramanyam M (2006). Indigenous health and socioeconomic status in India. PLoS Med. 3(10):e421.

Tiran D (2006). Integrated healthcare: herbal remedies for menopausal symptoms. Br. J. Nurs. 15(12):645-648.

Uniyal SK, Singh KN, Jamwal P, Lal B (2006). Traditional use of medicinal plants among the tribal communities of Chhota Bhangal, Western Himalaya. J. Ethnobiol. Ethnomed. 2:14.

Vidyasagar GM, Prashantkumar P (2007). Traditional herbal remedies for gynecological disorders in women of Bidar district, Karnataka, India. Fitoterapia. 78(1):48-51.

World Health Organization (2008). Commission on the Social Determinant of Health, Key Concepts. Geneva: WHO - Ref 35.

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Full Length Research Paper

Genetic variability studies of Valencia groundnut varieties for late leaf spot (*Phaeoisariopsis personata*) resistance

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The study was initiated to determine the genetic variability of late leaf spot (LLS) resistance among segregating generations of Valencia groundnut varieties. Crosses were made between NuMex-M_3 × ICGV-SM 02501, Valencia C × ICGV-SM 02501, Redbeauty × ICGV-SM 03590 and Valencia C × SGV-07009 parental lines and the resulting generations (F_1, F_2, BC_1P_1 and BC_1P_2,), along with parents for each cross, were evaluated for LLS resistance on a 1-9 scale under natural conditions in a randomized complete block design (RCBD) with three replications. Analysis of variance was performed for generations of each cross, coefficients of variation and heritability were estimated for all crosses except for the Valencia C × SGV-07009 cross. Three crosses showed highly significant differences among generations for LLS resistance ($P \leq 0.05$). The three crosses exhibited moderate to high levels of genotypic coefficient of variation (GCV) (15.43 to 23.13 %) and phenotypic coefficient of variation (PCV) (16.89 to 28.82%). The exception was the Redbeauty × ICGV-SM 03590 cross which showed low (9.50%) GCV. Broad-sense heritability (h^2_b) estimates for LLS disease scores were moderate to high (32 to 64%) for the three crosses. The results reveal substantial variation for LLS resistance in generations of these crosses indicating that the trait under study was heritable.

Key words: Valencia groundnut, *Arachis hypogeae,* late leaf spot, resistance, genetic variability.

INTRODUCTION

In Uganda, groundnut (*Arachis hypogeae* L) is the second most important legume crop after common beans (*Phaseolus vulgaris* L.) (UBOS, 2010) grown in all parts of the country (UBOS, 2010; Okello et al., 2013). The production volume gradually increased from about 130,000 tons on 216,000 ha in 2003 to over 185,000 tons on 253,000 ha in 2009 (FAO, 2009). As a legume, groundnut improves soil fertility by fixing nitrogen (Janila et al., 2013), and therefore, requires fewer inputs making it ideal for cultivation by resource poor farmers (Smartt, 1994). In addition it is well adapted to the hot, semi-arid conditions of Uganda (Okello et al., 2010; UBOS, 2010). As a cash crop, groundnut gives relatively high returns for limited land area. Nutritionally, groundnut kernels are a rich source of energy and a principal source of protein (Asibuo et al., 2008; Jambunathan, 1991; Shilpa et al., 2013). Groundnut is also a very good source of minerals (calcium, magnesium and iron) and vitamins (B1, B2 and Niacin) (Sigh and Diwakar, 1993). In addition, in many countries groundnut hay is used for fodder (Ozyigit and Bilgen, 2013), and the shells used for fuel (Janila et al., 2013).

The low productivity of the crop is ascribed mainly to foliar diseases of which late leaf spot (LLS) caused by *Phaeoisariopsis personata* (Berk. and Curtis) is said to be the most devastating fungal disease accounting for over 60% yield loss of Valencia groundnut in Uganda (Mugisha et al., 2004). According to Kalule et al. (2010), all Valencia varieties in Uganda are susceptible to LLS disease, and yet they are preferred by farmers for their early maturity attribute (Okello et al., 2010; 2013), by consumers for their sweet taste (Pattee et al., 2001) and by traders for their high oil content (Kaaya and Warren, 2005). Effective chemical control is heavily reliant on multiple fungicide applications (Jordan et al., 2012), which is costly for our resource poor farmers and may not be economical in our rain-fed agriculture (Page et al., 2002). The deployment of resistant cultivars is the most viable option to control LLS disease in groundnut, which could be effective in decreasing the production costs, improving product quality and reducing detrimental effects of fungicides on ecosystems. It is for these reasons that breeders choose to exploit the available genetic resources through plant genetic improvement techniques. However, little has been achieved due the lack of adequate information on genetic variability of LLS resistance on the available Valencia breeding materials, which makes genetic improvement of the crop difficult. In addition, the quantitative nature of LLS resistance (Dwivedi et al., 2002; Upadhyay et al., 2009; Khedikar et al., 2010), suggests that resistance is rather complicated, which could make direct selection for LLS resistance challenging in the breeding program. Information on the coefficients of variation and heritability helps to know whether the observed variability in the available material is due to genotype or environmental factors. Moderate PCV and GCV have been reported for LLS resistance in 28 F2 populations involving eight parents by Vishnuvardhan et al. (2012). In 2008 Khedikar reported high PCV (21.71 to 33.55) and moderate to high GCV (14.46 to 24.76) for LLS disease scores under natural conditions. Kumari (2008) observed high PCV (29.96 to 36.07) and GCV (27.71 to 32.96) for LLS resistance. Anderson et al. (1991) observed low to high broad sense heritability estimates for components of resistance to LLS disease. Vishnuvardhan et al. (2012) reported high and Khedikar et al. (2010) also reported high to very high (40.87 to 82.81%) h^2_b of LLS resistance in groundnut. However, Falconer and Mackay (1996) concluded that heritability values depend on the structure of the population and environmental conditions where the materials are evaluated. In this study, GCV, PCV and broad-sense heritability (h^2_b) for LLS resistance were estimated using Valencia breeding populations to generate more information to be used in suggesting a breeding program strategy for developing LLS resistant groundnut genotypes.

MATERIALS AND METHODS

The research was conducted at the National Semi-Arid Resources Research Institute (NaSARRI) of the National Agricultural Research Organization (NARO) located 01^0 30' 00" N and 33^0 33' 00" E in Serere district, Uganda. This location represents a humid and hot climate that receives an annual rainfall of 1,000-1,200 mm. In the study, groundnut genotypes with varying levels of resistance to LLS were used (Table 1). The genotypes had been characterized for resistance to LLS by the Groundnut Improvement Program at NaSARRI.

Generation of first filial generations (F_1 progenies)

Valencia lines Valencia C, NuMex-M_3 and Redbeauty were used as female (susceptible lines) while SGV- 07009, ICVG-SM 03590 and ICGV-SM 02501 were the male parents (resistant lines). In July 2011, three seeds from each of the parents were planted in plastic pots of diameter 45 cm and height 15 cm containing garden soil. The parental lines were grown in a glass house. Staggered planting of parents was done where the male parents were planted one week earlier than the female parents in order to synchronize flowering and to ensure continuous availability of flowers and floral buds for making crosses. Plants were watered every two days until they reached physiological maturity.

At flowering, the female parents were emasculated with forceps in the evening, and crossings were made the following morning. Bi-parental mating design was employed where four crosses were made between NuMex-M_3 × ICGV-SM 02501, Valencia C × ICGV-SM 02501, Redbeauty × ICGV-SM 03590 and Valencia C × SGV-07009 parental lines. In each cross 15 female flowers were pollinated. At physiological maturity the pods of the parental lines and crosses (F_1s) were harvested separately, dried, and packed in labeled envelops, and stored.

Generation of F_1, F_2, BC_1P_1 and BC_1P_2 populations

In December 2011, 15 F_1 seeds generated from each

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Table 1. Botanical names, origin, pedigree, and response to late leaf spot (LLS) of six groundnut lines.

Genotype	Botanical Name	Pedigree	Country of origin	Response to LLS
Redbeauty	Valencia	Landrace	Uganda	Susceptible
Valencia C	Valencia	Selection from Colorado Manfredi	USA	Susceptible
NuMex-M_3	Valencia	Valencia C × ICGV 87157	USA	Susceptible
JL 24	Spanish	Selection from Taiwan EC94943	India	Highly susceptible
ICVG-SM O3590	Virginia	-	Malawi	Resistant
ICGV-SM 02501	Spanish	-	Malawi	Resistant
SGV 07009	Virginia	SGV 91707× Serenut 1	Uganda	Resistant

cross described above, along with their respective parents were grown in a glass house. The F_1 seed were planted alongside their respective parents to identify the successful crosses. The parents were also used to generate more F_1 seeds as described above. At flowering, five F_1 plants were selfed to generate F_2 seeds while five plants were backcrossed to susceptible parents (P_1) and five plants backcrossed to donor plants (P_2) to produce $BC_1P_1$and BC_1P_2 seeds, respectively. The parents of the respective crosses were used as male parents, and the F_1 generation as female parents in generation of BC_1P_1 and BC_1P_2 seeds.

Evaluation of the six generations of the four crosses

Field lay out

The generations of the four crosses were evaluated in the experimental field at NaSARRI, a hot spot for LLS disease. Six generations P_1, P_2, F_1, F_2 and BC_1P_1 and BC_1P_2 of each of the four crosses: NuMex-M_3 × ICGV-SM 02501, Valencia C × ICGV-SM 02501, Redbeauty × ICGV-SM 03590 and Valencia C × SGV-07009, were set in a RCBD in three replicates with 2-row-plots of ten plants each. The populations and parental lines were planted in the field at a spacing of 45 × 15 cm in June 2012, and the experiment was kept free of weeds throughout the cropping season.

Inoculation

To maximize LLS inoculum pressure under natural conditions, the spreader row technique was used. The groundnut line JL 24, which is highly susceptible to LLS was used as a source of inoculum. Spreader rows were planted after every two rows of test materials and at the border of the experiments to maintain the effective inoculum load. These rows were planted two weeks before planting the experimental materials.

Data collection

LLS disease severity scoring was done at 115 days after planting using a modified nine point scale (Subrahmanyam et al., 1995), where a score of 1 was rated as highly resistant (HR), 2-4 as resistant (R), 5-6 as moderately resistant (MR), 7-8 as susceptible (S), and 9 as highly susceptible (HS).

Statistical analysis

Analysis of variance

Data taken on the generations of each cross were subjected to ANOVA using GenStat version 13 software to test for the significance of the differences between the generations' means of each cross for the LLS disease scores. The ANOVA was based on the linear mathematical model: $Y_{ij}= \mu + r_i + g_j + e_{ij}$; where Yij= observed effect for i^{th} replication and j^{th} genotype, μ = grand mean of the experiment, r_i = effect of the i^{th} replication, g_i = effect of the j^{th} genotype, e_{ij} = residual effect. The generation means were compared using Fisher's protected least significant difference test at 5% level of probability (Payne et al., 2010).

Estimation of PCV, GCV, heritability and genetic advance

In order to determine PCV, GCV, heritability and genetic advance for LLS resistance, variance components (environmental and genotypic variances) were obtained following the method of Kearsey and Pooni (1996) for the three crosses (NuMeX-M_3 × ICGV-SM 02501, Valencia C × ICGV-SM 02501, and RB × ICGV-SM 03590).

Estimation of PCV and GCV: Both PCV (i) and GCV (ii) were estimated following the method suggested by Singh and Chaudhury (1985) and classified as described by Sivasubramanian and Menon (1973) as; low (0-10), medium (10-20) and high (20 and above).
(i) Phenotypic coefficient variation (PCV) = $(\sqrt{V_P}/\bar{X})*100$
(ii) Genotypic coefficient variation (GCV) = $(\sqrt{V_G}/\bar{X})*100$
Where, V_P= Phenotypic variance, V_G=Genotypic variance and $\bar{X}$=Grand mean of the character.

Estimation of broad-sense heritability: Variance components (environmental and genotypic) obtained above were used to determine broad sense heritability (h^2_b) (Kearsey and Pooni, 1996) in the three crosses NuMex-M_3 × ICGV-SM 02501, Valencia C × ICGV-SM 02501, andRB × ICGV-SM 03590 as detailed below.
Broad-sense (h^2_b) = $100[\sigma^2G(F_2)/V_{F2}]$
Where; $\sigma^2G(F_2)$= Genotypic variance in F_2 and V_{F2}= variance of F_2 generation.

Genetic advance (GA): Genetic advance (GA) was estimated following Singh and Chaudhury (1985) method as;

$GA = h^2_b \times k \times \sigma^2_p$

Where, h^2_b = broad sense heritability estimate, σ^2_p = Phenotypic standard deviation, K = Selection intensity at 5 % is equal to 2.06.

Genetic advance as percent of mean (GAM) was then determined as:

GAM % = $(GA/\bar{X})*100$

Where, $\bar{X}$= Grand mean of the trait, GA = Genetic advance.

Table 2. Results of LLS mean score for the six generations of the 4 crosses.

Generation	NuMeX-M_3 × ICGV-SM 02501	Valencia C × ICGV-SM 02501	Valencia C × SGV 07009	Redbeauty × ICGV-SM 03590
P_1 (S)	6.79±0.25^c	7.44±0.38^d	7.29±0.61^b	7.00±0.41^c
P_2(R)	3.42±0.18^a	3.40±0.16^a	8.36±0.31^b	3.50±0.50^a
F_1	3.50±0.50^{ab}	3.83±0.40^{ab}	7.52±0.78^b	4.50±0.50^a
F_2	5.33±0.88^b	5.22±0.40^c	5.00±0.38^a	4.60±0.40^a
BC_1P_1	5.25±0.75^b	4.75±0.48^{bc}	8.17±0.65^b	5.00±0.58^{ab}
BC_1P_2	4.75±0.63^{ab}	4.25±0.63^{abc}	5.25±0.37^a	4.50±0.50^a
MS	27.32	20.14	21.20	4.03
F	19.93**	20.80**	13.66**	4.35**
CV %	22.1	20.6	18.2	18.1

**=significant at P<0.01, S = susceptible, R = resistant, F_1= first filial generations F_2 = Second filial generations and $BC_1P_1$and BC_1P_2 backcrossed to susceptible parents (P_1) and donor parents (P_2), CV%=Coefficient of variation, F = Variation ratio, MS=Mean sum of square.

Table 3. Genetic parameters for resistance to LLS in groundnut.

CROSS	NuMex-M_3 × ICGV-SM 02501	Valencia C × ICGV-SM 02501	Redbeauty × ICGV-SM 03590
V_E	0.83	0.90	0.54
V_G	1.50	0.54	0.25
PCV	28.82	25.21	16.89
GCV	23.13	15.43	9.50
h^2_b(%)	64.00	37.00	32.00
$\bar{X}$	5.30	4.77	5.30
GA	1.13	0.67	0.22
GAM%	21.37	13.63	4.17

V_E=Environmental variance,V_G=Genotypic variance, PCV and GCV=Phenotypic and Genotypic Coefficient of Variation respectively,$h2_b$=Broad heritability, $\bar{X}$=Grand mean of all generations for each cross, GA=Genetic advance and GAM%=Genetic advance as percent of mean.

The Genetic Advance as percent of Mean (GAM %) was categorized following the procedure of Johnson et al. (1955) as low (0-10), medium (10-20) and high (20 and above).

RESULTS

Analysis of variance

The results of ANOVA and Fisher's protected least significant difference tests are shown in Table 2. The four crosses NuMex-M_3 × ICGV-SM 02501, Valencia C × ICGV-SM 02501, Redbeauty × ICGV-SM 03590, and Valencia C × SGV-07009 showed significant differences among generations for LLS scores (P≤0.01). The mean disease scores of the donor parents ICGV-SM 02501 and ICGV-SM 03590 were low while SGV-07009 had very high disease scores, which were similar to the susceptible parents: NuMex-M_3, Redbeauty and Valencia C. In all crosses except Valencia C × SGV-07009, the means of the parents (P_1 and P_2) showed a tendency to be more extreme and contrasting for LLS resistance. Therefore, the later cross was excluded for further analysis. Moderate to high levels of LLS resistance were observed in all populations of the crosses. In general, the backcrosses, BC_1P_1 and BC_1P_2 showed the mean LLS disease score close to their respective recurrent parents Table 2. The segregants in the F_2 generation of the crosses Valencia C × SGV-07009 , Valencia C × ICGV-SM 02501 and M3 × ICGV-SM 02501 showed moderate severity for LLS disease scores, while that of Redbeauty × ICGV-SM 03590 were highly resistant to LLS.

Estimation of PCV, GCV and heritability

The results demonstrating PCV and GCV, heritability and genetic advance estimates for resistance to LLS are presented in Table 3. The PCV estimates were high in NuMex-M_3 × ICGV-SM 02501(28.82 %) and Valencia C ×

ICGV-SM 02501 (24.51 %) crosses and moderate in Redbeauty× ICGV-SM 03590 (16.89 %). The genetic coefficient of variation (GCV) estimates were high in cross NuMex-M_3× ICGV-SM 02501(23.13 %), moderate in Valencia C × ICGV-SM 02501(15.87 %) and low in cross Redbeauty × ICGV-SM 03590 (9.50 %). Broad-sense heritability estimates for LLS disease score were 32, 37 and 64%, respectively, for Redbeauty × ICGV-SM 03590, Valencia C × ICGV-SM 02501 and NuMex-M_3 × ICGV-SM 02501. Genetic Advance as percentage of Mean (GAM) were low in the cross between Redbeauty × ICGV-SM 03590 (10.93%), moderate in Valencia C × ICGV-SM 02501 (19.43%) and high in NuMex-M_3× ICGV-SM 02501 (38.19%).

DISCUSSION

The ANOVA for the 4 crosses showed highly significant differences (P≤ 0.01) among generations for late leaf spot resistance (Table 2), suggesting presence of genetic variability for LLS disease score in the generations. Variability for LLS resistance was also reported by Vishnuvardhan et al. (2012) in experimental materials that comprised of 28 F2 populations. In the present study genotypes ICVG-SM 02501 and ICGV-SM 03590 showed high resistance to LLS and are recommended for use in breeding program as sources of resistance to late leaf spot. An earlier report by Kalule et al. (2010) demonstrated that these lines were the best parents for LLS resistance. Moderate to high levels of LLS resistance was observed in the populations of the 4 crosses (Table 2), indicating that the trait under study was heritable. The results partly agree with that of John et al. (2008) who reported moderate incidence of LLS in F2 population of the Kadiri-3 × ICGV-88083. Kornegay et al. (1980) also observed minimal leaf defoliation in F1 and F2 generations of the cultivated Virginia.

The mean of F_1s of NuMex-M_3 × ICGV-SM 02501, Valencia C × ICGV-SM 02501 and Redbeauty × ICGV-SM 03590 crosses tended towards the mean of the ICGV-SM 02501 and ICGV-SM 03590 resistant parents, respectively (Table 2), indicating mid-parent heterosis in these crosses. In groundnut however, commercial production of F_1 seed is not feasible since it's predominately self-pollinated. According to John et al. (2012), heterotic crosses in self-pollinated crops help breeders to select appropriate crosses that could lead to desirable transgressive segregants in advanced generations. Therefore, breeding methods such as recurrent selection may be used in exploitation of such heterosis in future breeding programs for these crosses.

Several reports indicate that resistance to LLS in groundnut is controlled by several recessive genes (Nevill 1982; Dwivedi et al., 2002; Upadhyay et al., 2009; Khedikar et al., 2010), though, the F_1's in the present study exhibited partial resistance. Walls and Wynne (1985) concluded that partial resistance in F_1 could not be explained solely by completely recessive genes. They suggested that modifier genes were affecting the phenotypic expression of genes at loci controlling resistance. Anderson et al. (1990) also reported that recessive as well as modifier genes may be involved in resistance to LLS disease in groundnut.

The donor line SGV-07009 was highly susceptible to LLS, in spite of the fact that segregating populations F_2 and BC_1P_1 of the cross Valencia C × SGV-07009 were moderately resistant (Table 2). Natarajan et al. (2001) reported that crosses involving susceptible parents may tend to produce resistant progenies with stable resistance due to additive genetic action. Babu (2010) recommended that such transgressive segregants that arise from the susceptible parents on both sides can also be used as potential genetic stocks in resistance breeding programs. However, this cross was excluded for further analysis.

Moderate to high levels of genetic coefficients of variability **(**GCV) (15.43 to 23.13) and phenotypic coefficients of variability **(**PCV)(16.89 to 28.82) were noticed for LLS resistance in all the three crosses, except for the cross Redbeauty× ICGV-SM 03590 which showed low GCV (9.50) Table 3. The results are comparable with Vishnuvardhan et al. (2012) observation of moderate PCV (19.04) and GCV (16.48). The results of the study also partly agree with those of Khedikar et al. (2010) which indicated high (21.71 to 33.55) PCV, Khedikar, (2008) which indicated moderate to high (14.46 to 24.76) GCV and Kumari (2008) which indicated high PCV (29.96 to 36.07) and GCV(27.71 to 32.96) for late leaf spot resistance. High PCV and moderate to high levels of GCV revealed high magnitude of heritable variation for LLS resistance in these crosses.

In the current study, high GCV (23.13) was exhibited in the cross NuMex-M3 × ICGV-SM 02501. A high GCV indicated that the character had high variability which can be attributed to genotype and with very little effect of the environment. According to Oyiga and Iguru (2011), when the magnitude of GCV is higher, it indicates that the genetic component is the major contributor to the total variance of the trait under study. High PCV and GCV of a trait may result in high heritability which suggests that the improvement of this trait by simple selection method could be possible. Vishnuvardhan et al. (2012) concluded that high GCV may indicate a predominant role of additive gene actions and amenability for phenotypic selection in early generations.

Although the GCV values revealed the extent of genetic variability present in the genotypes for LLS resistance, GCV values are not enough to assess the level of genetic variability among the genotypes. Genetic variation could further be explored with help of heritability estimates, which measures the heritable portion of the total variation. In the present study moderate to high h^2_b revealed the existence of inherent variability among the genotypes, which is more useful for exploitation in selection and hybridization programs. All the three crosses in the present study showed moderate to high h^2_b (32-64%) and low to high GAM (4.17-21.37%). Moderate to very high h^2_b (40.87 to 82.81) estimates were also reported for leaf spot disease severity in groundnut Khedikar et al. (2010) and Kumari (2008) also report very high h^2_b (83.50 to 85.50). Vishnuvardhan et al. (2012) reported moderate GCV (16.48), high h^2_b (74.91) and GAM (29.38) while Kumari (2008) observed high GCV (27.71 to 32.96), h^2_b (83.50 to 85.50) and GAM (52.78 to 62.05) for late leaf spot severity. The discrepancy of the results is not unexpected because such quantitative traits are often affected by several environmental factors and the genetic background of the parental materials. Falconer and Mackay (1996) concluded that heritability values depend on the population and environmental conditions in which the materials are evaluated.

High h^2_b (64.41 %) estimates were observed in the cross NuMex-M_3 × ICGV-SM 02501 indicating a high response to selection due to reduced environment influence thereby validating the results obtained with the high GCV value for this cross. Anderson et al. (1986) also observed high heritability for LLS resistance and concluded that individual plant selection for LLS would be effective in early generations. Moderate h^2_b estimates were observed in Redbeauty × ICGV-SM 03590 (32 %) and Valencia C × ICGV-SM 02501 (37 %) crosses suggesting a high influence of the environment on the trait in these crosses. The high environmental variation could have been a result of variation in relative humidity within the micro-climates. Thus, selection of genotypes from initial generations by LLS disease scores in these crosses may be difficult. Singh (1993) concluded that low to moderate heritability estimates makes selection considerably difficult or virtually impractical due to the masking effect of the environment on the genotypic effect. Furthermore, LLS resistance is polygenically controlled, and cumulative environmental effects on this polygenically controlled trait could have given poor heritabilities for this trait. In such cases simple selection may not be rewarding. Breeding efforts to increase resistance will require good control over environmental variance. Adequate experimental design, accurate phenotyping are key interventions that could increase the heritability of such a polygenic trait.

Heritability estimates when coupled with genetic advance provides a better prediction of expected gain under selection instead of heritability alone. The estimates of genetic advance help in understanding the type of gene action involved in the expression of various polygenic characters (Singh and Narayanan, 1993). High heritability (h^2_b) (64%) coupled with high (21.27) genetic advance was observed for LLS resistance in the NuMex-M_3 X ICGV-SM 02501 cross, indicating significant role of additive gene action for its inheritance. Therefore, simple selection methods would be effective for improvement of LLS resistance from this cross. The results are comparable with reports of Vishnuvardhan et al. (2012), which indicated high GA for LLS resistance. Moderate (32 to 37%) heritability (h^2_b) estimates along with low to moderate (4.17 to 13.63%) genetic advance was observed in Valencia C × ICGV-SM 02501 and Redbeauty × ICGV-SM 03590 crosses, which indicated that additive and non-additive gene actions had a role in the inheritance. The successful breeding methods will be the ones, which exploit additive and non-additive gene effect such as recurrent selection (Nidagundi et al., 2012) and use of biparental mating (Dabholkar, 1992; Soomro et al., 2010).

Conclusions

Based on the observed results, it can be concluded that a considerable amount of genetic variation for LLS resistance existed among the segregating generations of the Valencia groundnut varieties, which can guarantee substantial improvement through selection. However, the amount of variation depended on the genetic back-grounds of the parents that were used in the study. The best strategy for obtaining LLS resistant genotypes is for selection to be carried out in initial inbreeding generations for the cross between NuMex-M3× ICGV-SM 02501, followed by selection in the following generations with higher inbreeding levels in other crosses.

Conflict of Interest

The authors have not declared any conflict of interest.

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REFERENCES

Anderson WF, Beute MK, Wynne JC, Wongkaew S (1990). Statistical procedures for assessment of resistance in a multiple foliar disease complex of peanut. Phytopathol. 80:1451-1459.
Anderson WF, Holbrook CC, Wynne JC (1991). Heritability and early-generation selection for resistance to early and late leaf spot in peanut. Crop Sci. 31:588-593.
Anderson WF, Wynne JC, Green CC (1986). Potential for incorporation of early and late leaf spot resistance in peanut. Plant Breed. 97:163-170.
Asibuo JY, Akromah R, Safo-Kantanka O, Adu-Dapaah HK, Ohemeng-Dapaah S, Agyeman A (2008). Chemical composition of groundnut,

Arachis hypogaea (L) landraces. Afr. J. Biotechnol. 7:2203-2208.
Babu C (2010). Pre-breeding in sugarcane (*Saccharum* sp. hybrids) for red rot resistance and economic traits. Electron. J. Plant Breed. 1:1024-1034.
Dabholkar AR (1992). Elements of Biometrical Genetics. Concept Publishing Company,New Delhi 110059, South Asia. pp. 38-165.
Dwivedi SL, Pande S, Rao JN, Nigam SN (2002). Components of resistance to late leaf spot and rust among interspecific derivatives and their significance in a foliar disease resistance breeding in groundnut *Arachish ypogaea* (L.). Euphytica, 125:81-88.
Falconer DS, Mackay FCT (1996). Introduction to Quantitative Genetic 4th Edition Longman Group Limited. pp.108-183.
FAOSTAT (2009). Crop Productivity. http://faostat.fao.org.
Jambunathan R (1991). Groundnut quality characteristics. Pages 267-275 *in* Uses of tropical grain legumes: Proceedings of a Consultants Meeting, 27 -30 Mar 1989 (Patancheru:ICRISAT) 267-275.
Janila P, Nigam SN, Pandey MK, Nagesh P, Varshney RK (2013). Groundnut improvement: use of genetic and genomic tools. Plant Sci. 4:1-12.
John K, Reddy RP, Reddy KH, Sudhakar P, Reddy NPE (2012). Identification of best heterotic crosses for yield and water use efficiency traits in groundnut (*Arachishypogaea* (L.). J. Plant Breed. Crop Sci. 4:17-24.
John KRP, Vasanthi O, Venkateswarlu T, Krishna M, Naidu PH (2008). Genetic analysis of pod yield and resistance to biotic stresses in groundnut (*Arachis hypogaea l.*). Legume Res. 31:227-229.
Johnson HW, Robinson HF, Comstock RE (1955). Estimates of genetic and environmental variability in soybeans. Agron. J. 47:314-318.
Jordan DL, Brandenburg RL, Brown AB, Bullen GS, Roberson GT, Shew B, Spears FJ (2012). Peanut Information. North Carolina Cooperative Extension Service, College of Agriculture & Life Sciences North Carolina State University.
Kaaya NA, Warren HL (2005). A Review of Past and Present Research on Aflatoxin in Uganda. Afr. J. Food Agric. Nutr. Dev. 5:1-3.
Kalule D, Deom C, Puppala N (2010). Screening groundnut accessions for Rossette and Leaf spot Diseases in Uganda. Annual Meetings of ASA, CSSA, SSA, Long Beach, CA – Oct 31 – Nov (abstr.).
Kearsey JM, Pooni SH (1996). The genetic Analysis of quantitative Traits. 1stEdn. Chapman and hall.
Khedikar PY (2008).Molecular Tagging and Mapping of Resistance to Late Leaf Spot and Rust in Groundnut (*Arachis hypogaea*L.). phd Thesis University of Agricultural Sciences, Dharwad.
Khedikar YP, Gowda MVC, Sarvamangala C, Patgar KV, Upadhyaya HD and Varshney RK (2010). A QTL study on late leaf spot and rust revealed one major QTL for molecular breeding for rust resistance in groundnut (*Arachis hypogaea* L.). Theor. Appl. Genet. 121:971-984.
Kumari V (2008). Morphological and Molecular Characterization of Induced Mutants in Groundnut.Thesis Degree of Master of Science Agriculture. University of Agricultural Sciences, Dharwad.
Mugisha J, Ogwalo R, Ekere W, Ekiyar V (2004). Adoption of IPM Groundnut production Technologies in Eastern Uganda. Afr. Crop Sci. J. 12:383-391.
Natarajan US, Balasundaram N, Ramana Rao TC, Padmanaban P, Mohanraj D, Karthigeyan S, Damodaran S (2001). Role of *Saccharum spontaneum* in imparting stable resistance against sugarcane red rot. Sugarcane Int. pp.17-20.
Nevill DJ (1982). Inheritance of resistance to *Cercosporidium personatum*in groundnuts: A genetic model and its implications for selection. Oleagineux, 37:355-366.
Nidagundi JM, Patil SS, Salimath PM, Kajjidoni ST, Patil BC, Hegde MG (2012). Genetic analysis of seed cotton yield and its component traits in *Gossypium hirsutum*L. Karnataka J. Agric. Sci. 25:260-261.
Okello DK, Biruma M, Deom CM (2010). Overview of Groundnut research in Uganda: Past, Present and Future. Afr. J. Biotechnol. 9:6448-6459.
Okello DK, Monyo E, Deom CM, Ininda J, Oloka HK (2013). Groundnuts production guide for Uganda: Recommended practices for farmers. National Agricultural Research Organisation, Entebbe. ISBN: 978-9970-401-06-2
Oyiga BC, Iguru MI (2011). Genetic variation and Contributions of same floral traits to Pod yield in Bambara Groundnut (Vigna subterranean L.Verdc) Under Two Cropping Seasons in the Derived Savana of South-East Nigeria. Int. J. Plant Breed. 1:58-63.
Ozyigit Y, Bilgen M (2013). Forage Potential of some Groundnut (*Arachis hypogaea L.*) Cultivars. Rom. Agric. Res. 30:1-6.
Page WW, Busolo-Bulafu CM, vander Merwe PJA, Chancellor TCB (2002). Groundnut manual for Uganda: Recommended groundnut production practices for smallholder farmers in Uganda. Chatham, UK: Natural Resources Institute. pp. 1-12.
Pattee HE, Isleib TG, Gorbet DW, Giesbrecht FG, Cui Z (2001). Parental selection in breeding for roasted peanut flavor quality. Peanut Sci. 28:51-58.
Payne R, Harding WSA, Murray DA, Soutar DM, Baird DB, Glaser AI, Channing IC, Welham SJ, Gilmour AR, Thompson R, Webster R (2010). A Guide to Anova and Design in GenStat. 13thEdn. VSN International ltd. pp. 1-103
Shilpa k, Sunkad G, Kurella S, Marri S, Padmashree K, Jadhav DR., Sahrawat K,Mallikarjuna N (2013). Biochemical Composition and Disease Resistance in Newly Synthesized Amphidiploid and Autotetraploid Peanuts. Food. Nutr. Sci. 4:169-176.
Singh BD (1993). Plant Breeding 5thEdn, Kalyani Publshers, Rajender Nagar, pp. 102,104.
Singh F, Diwakar B (1993).Nutritive Value and Uses of Pigeonpea and Groundnut. Human Resource Development Program. ICRISAT. Patancheru, Andhra Pradesh 502-324, India.
Singh P, Narayanan SS (1993). Biometrical techniques in plant breeding. Kalyani, Publishers New Delhi.
Singh RK, Chaudhary BD (1985). Biometrical Methods in Quantitative Analysis. Kalayani Publishers. New Delhi. pp. 318.
Sivasubramanian S, Menon M (1973). Heterosis and inbreeding depression in rice. Madras Agric. J. 60:1139.
Smartt J (1994). The groundnut in farming systems and the rural economy a global view.In: The Groundnut Crop: A Scientific Basis for Improvement. J. Smartt, ed. Chapman & Hall, London, pp. 664-699.
Soomro ZA, Kumbhar MB, Larik AS, ImranM, Brohi SA (2010). Heritability and Selection Response in Segregating Generation of Upland Cotton. Pak. J. Agric. Res. 23:25-29.
Subrahmanyam P, McDonald D, Waliyar F, Reddy LJ, Nigam SN, Gibbons, RW, Rao VR., Singh AK, Pande S, Reddy PM, Rao PVS (1995). Screening methods and sources of resistance to rust and late leaf spot of groundnut. Information bulletin No: 47 ICRISAT.
Uganda Bureau of Statistics (UBOS) (2010). Uganda Census of Agriculture 2008/2009. Volume IV: Crop Area and Production Report.
Upadhyay RK, Mukherji KG, Chamola BP, Dubey OP (2009). Intergrated pest and disease management. A.P.H Cooperation. New Delhi.
Vishnuvardhan KM, Vasanthi RP, Reddy KHP, Reddy BV (2012).Genetic variability studies for yield attributes and resistance to foliar diseases in groundnut *(Arachish hypoge*al.). Int. J. Appl. Biol. Pharm. Technol. 3:390-394.
Walls SB, Wynne JC (1985). Combining ability for resistance to *(Cercosporidium personattum)* for five late leaf spot-resistant peanut germplasm lines. *Oleagineux*, 40:389-396.

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Comparative response of catechin levels in drought tolerant and drought susceptible tea clones (*Camellia sinensis*) (L) O. Kuntze under different moisture regimes

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In tea (*Camellia sinensis*) water stress generally affects the content of various plant secondary metabolites including catechins. The objective of the study was to evaluate the effects of different soil moisture content on the catechin levels of various tea clones. We found out that variation of soil water content and accumulation of catechins were strongly correlated. The experiment was conducted in an open field with the drought tolerant clones namely; SFS150, TRFK 303/577, and drought susceptible clones; TRFK 6/8, TRFK 12/9, TRFK 301/4, TRFK 31/11, S15/10, TRFK 7/9, TRFK 31/8, and BBK 35. During the cold and wet periods, the effect of plant water content on catechin level was not clearly expressed. However, significant clone × soil water content interactions ($p \leq 0.05$) were observed for all clones during the dry and hot periods. This observation indicated that declining plant water content (PWC) due to soil moisture stress reduced catechin levels. It was concluded that variation of catechin in various tea clones over different soil water content could be of great significance in evaluating water stress tolerance ability of tea clones.

Key words: Catechins, clones, water content, dry and hot periods.

INTRODUCTION

Water stress in tea plant due drought effects results in physiological, biochemical and morphological changes such as reduction of leaf water potential, photosynthesis and stomatal conductance (Bota et al., 2004; Zobayed et al., 2007; Damayanthi et al., 2010). Proline and abscisic acid (ABA) accumulate in higher concentrations in response to water stress, which leads to maintenance of turgor potential (Mayer, 2006). That different forms of stress affect the content of various plant secondary metabolites including polyphenols has been reported (Borland et al., 2009; Cherotich et al., 2013) but variation in tea polyphenolics over different seasons has not been quantitatively evaluated.

The study was conducted to check whether the drought

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tolerant and susceptible clones display a distinct pattern of variation in catechin levels when subjected to different water regimes and whether the pattern agrees with the available data on the drought tolerance ability of the studied clones with a view of using the principle in selection of clones.

MATERIALS AND METHODS

Experimental site

The experiment was located in Field 12C at Timbilil estate, Tea Research Foundation of Kenya (0^0 22' S; 35^0 21' E), altitude; 2178 m above mean sea level. The topography of the area is steeply dissected with an average slope of 30 degrees (Callander and Woodhead, 1981).

Physicochemical characteristics of experimental site

The soil in the study area is a fine mixture of clay of kaolinite type (75-85%) and organic matter (30%) (Othieno et al., 1992) hence, it has many properties in common with those of other tea growing areas of Kenya. It is highly weathered, leached and acidic, pH 4.5 soil conditions in which tea grows best (Othieno et al., 1992). The soils are deep and well drained with crumby surface soil structure grading to a moderate aggregate structure in the sub-soil with many pore spaces (50%) making it ideal for tea growth (Watson, 1986). The surface soil colour is dark brown grading to strong brown in the moist sub soil.

Plant materials

Ten contrasting cultivars of tea in terms of drought tolerance and superior beverage quality attributes were selected from the existing tea bushes (average age 28 years). These were; drought tolerant clones: SFS 150 and TRFK 303/577[control], and drought susceptible clones: TRFK 6/8, TRFK 301/4, TRFK 12/19, TRFK 31/11, BBK 35, S15/10, TRFK 7/9, and TRFK 31/8 [control]

Experimental design and treatments

The experiment was superimpossed on Field 12C with mature, fully grown tea bushes established in 1983 in what was a virgin forest land. The exprimental area was arranged in 30 randomized blocks each measuring 225.5 m^2 surrounded by single guard row. The effective plot area consisted of 100 bushes with spacing of 1.2 m between rows and 0.75 m between plants. The effective plot area was subdivided to form three replicates each having 30 plants.

Biometric measurements

For the biometric measurements, mature, fully grown and healthy leaves on the plucking table were selected. The physiological and biochemical parameters that were evaluated were; total catechin content and leaf water potential. Soil moisture content was also measured to study the responses of tea plants to soil water content. All the measurements were made between 9.00 a.m. and 2.00 pm at intervals of 1 h.

Total catechin content (TCC)

Leaves weighing 300 g (the terminal two leaves and bud) were randomly collected from each plot during the cold June to August and wet September to November periods when the conditions are relatively warm and wet. The last sampling was done towards the end of the dry and hot period December-March when tea plants were experiencing severe water stress.

Preparation of extracts

Sampled fresh green leaves from each plot were carefully steamed in a pressure cooker for one minute then placed in the withering bay and left to dry for twelve hours. The dried samples were processed by crush, tear and curl (CTC) then ground using a blender into fine powder, then sealed in paper bags (with aluminium foil lining) and safely stored in dark dry environment of 4°C awaiting the analysis.

Analysis of catechins

Estimation of catechins was determined according to the procedures of Folin Ciocalteu method (Piendl and Biendl, 2000). Fresh leaves (0.5 g) were homogenized in 5 ml of 70% methanol using a chilled pestle and mortar with subsequent centrifugation at 4000 revolutions per minute for 20 min. From the solution 10 ml was pipetted and mixed with Gallic acid standard solutions (0.1 ml) and 50 g anhydrous Gallic acid transferred into the reagent tube. Folin Ciocalteu phenol reagent was then added to each tube. Within five minutes from adding Folin Ciocalteu phenol reagent, 5.0 ml of sodium carbonate solution was added to stabilize the material and allowed to stand for 2 h at room temperature for completion of the reaction. The amount of catechins in the test sample was calculated from a standard curve generated using Gallic acid and then expressed as the amount of Gallic acid equivalent. A best-fit linear calibration graph from the mass of anhydrous gallic acid standards was constructed against the Gallic acid standard optical densities. The contents of catechins in the leaf were then expressed as percentages of the mass of sample dry matter.

Soil moisture content (SMC)

Soil moisture content of the root zone at 60 cm depth was measured along with physiological parameters of tea, leaf water potential and catechin levels. Soil was augured at 60 cm depth and the soil moisture content was determined using time-domain reflectometry (TDR) soil moisture meter (Trime FM-2, Eijkelkamp Agrisearch Equipment Giesbeek, and the Netherlands)

Leaf water potential (LWP)

Leaf water potential was determined periodically in different study periods, that is during wet and cold season (May-August), hot and wet season (October-December), 2011 and then again during the dry and hot period between January and March, 2012. Leaf water potential was measured between 10.00 am and 2.00 pm at an interval of 1 h on the entire leaf by observing the presence of water on the cut surface of the leaf petiole using a pressure chamber (Soil Moisture Equipment Corp., Santa Barbara, California) (plate 3.3). Two mature leaves (2^{nd} and 3^{rd}) and a bud were randomly selected from each plot for leaf water potential measurements. The leaf was enclosed in a reflective plastic bag for 1 h to suppress transpiration and allow stem water potential to equilibrate with leaf water potential (Dale, 2006; Kwach, 2011). The leaf was cut (in slanting manner) and enclosed in the pressure chamber with the cut end protruding through a rubber stopper which is used to seal the chamber. The pressure in the chamber was gradually increased

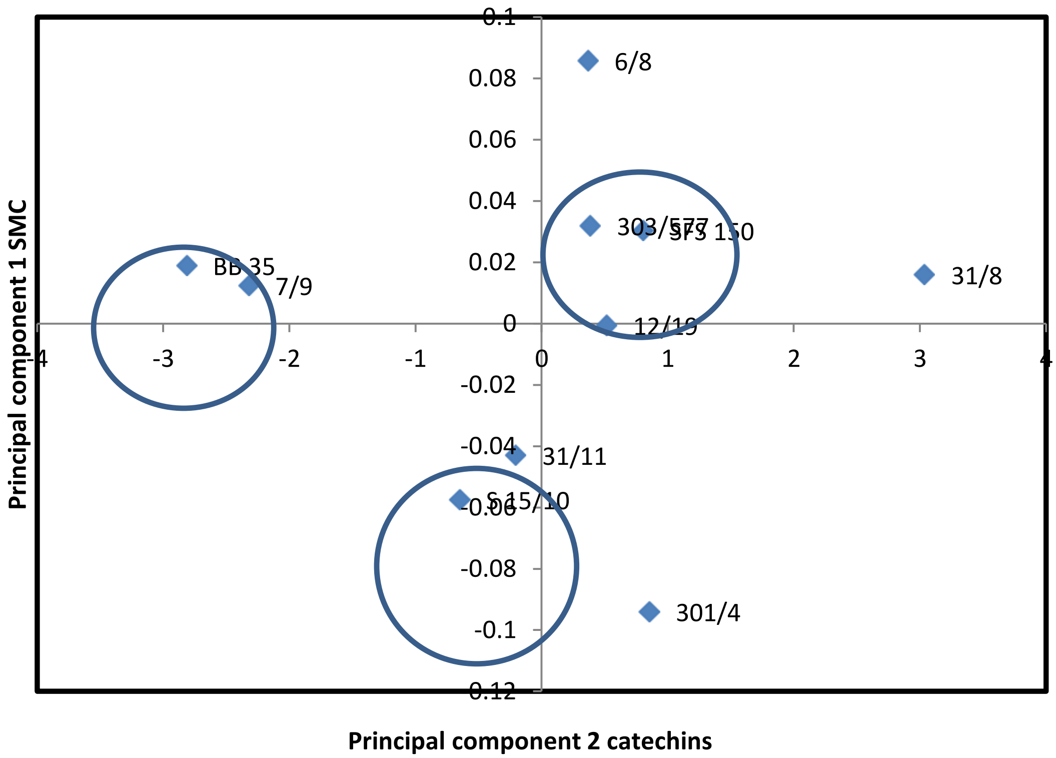

Figure 1. Principal component analysis of catechins for 10 clones across three seasons.

until the sap appeared at the end of the xylem vessels. After the pressure was recorded, the sap was released through an outlet valve and the sample removed (Phene et al., 1990).

Meteorological data

Meteorological data were recorded using instruments installed in Tea Research Foundation of Kenya (TRFK) weather. All instruments were mounted 1 m above the soil. Hourly records, of minimum and maximum temperatures, total radiation and rainfall were taken on a daily basis. The total solar irradiance during the period of study was measured using Gunn Bellani Pyronometer placed at 20 cm above the plucking table. The rate of evaporation was also recorded using Class 'A' pan.

Statistical analysis

Principal components analyses (PCA) were used to draw out the effects and interactions of the different soil moisture content and catechin levels. All one-way ANOVAs were accompanied by mean separation by Duncan Multiple Range Test (DMRT) using the SAS version 8.0 statistical packages (SAS Institute Inc: 1999).

RESULTS AND DISCUSSION

Variation of catechin content across three water regimes

Data were treated with principal component analysis (PCA) (Figure 1) to determine influence of soil water content on catechin accumulation and establish stable clones across the seasons (the different water regimes). The analysis placed the clones into three groups corresponding to stability of catechin content across the three seasons (wet, hot and wet, dry and hot). The first group includes 25% of the clones all known to be possessing moderate to high water stress tolerant characteristics. The rest of the clones (75%) are known to be susceptible to water stress. Clones placed on the side with positive value indicate stable content of catechins across all the seasons. A negative value indicates instability of catechin content in a clone across the three seasons thus indicating low tolerance ability to water stress.

Catechin contents of clones 303/577, 6/8, 31/8 and SFS 15/10 appearing to the right side of vertical ordinate showed little interaction across the three seasons depicting stable characteristic while those to the left are quite unstable across the three periods indicating moderate influence of soil water content on catechin accumulation in these clones whilst those below the horizontal axis are also very unstable suggesting high interaction between soil water content and clonal response.

Clones that have common parental linkage such as 303/577, 31/8 and 6/8 were clustered into the same ordinate. Therefore, it allows the hypothesis that clones that are closely related have almost the same

Table 1. Comparison of leaf water potential (Leaf) and catechins contents in seasons I, II and III at TRFK, Kericho

Clone	Season I[Cool and wet] June-August		Season II[Warm and wet] September-November		Season III [Dry and hot] December-February	
	Ψ_{Leaf} (MPa)	Catechins (%)	Ψ_{Leaf} (MPa)	Catechins (%)	Ψ_{Leaf} (MPa)	Catechins (%)
TRFK 31/8	-4.60	0.18	-6.73	0.16	-16.03	0.20
TRFK301/4	-4.87	0.15	-6.53	0.13	-18.37	0.09
TRFK311/11	-4.40	0.13	-6.53	0.11	-18.93	0.15
TRFK303/577	-4.00	0.23	-6.00	0.22	-17.17	0.24
TRFK 6/8	-3.73	0.17	-6.03	0.18	-18.73	0.21
TRFK 7/9	-4.80	0.11	-6.0	0.07	-17.47	0.17
BBK35	-4.40	0.10	-6.40	0.08	-16.50	0.13
SFS150	-4.67	0.22	-6.20	0.22	-15.80	0.24
S15/10	-5.9	0.11	-6.87	0.14	-18.97	0.16
TRFK 12/19	-4.27	0.12	-6.40	0.09	-16.50	0.15
F	s	s	s	s	s	s
CV (%)	7.54	22.83	4.76	31.23	3.04	34.23
LSD	P≤0.05		P≤0.05		P≤0.05	

s, Significant; MPa. Megapascals; LSD. Least significant difference; cv., coefficient of variation. Measurements were made between 10.00 am and 1.00 pm at intervals of an hour.

response to the environment.

Change in Leaf water potential (Ψ_{Leaf}) in relation to catechins contents

The changes in leaf water potential (LWP) of different clones are presented in (Table 1). Leaf water potential declined over dry period in all cases but the tolerant clones showed the least change. In general, leaf water potential in susceptible clones fell to almost similar low mean values during dry periods. These results suggest that clones with tolerant characteristics compared to the susceptible clones have better mechanism of maintaining of high leaf water status through effective the regulation of catechins levels.

At the lowest moisture levels, the drought tolerant clones TRFK 303/577 and SFS 150 maintained significantly higher (P≤0.05) leaf water potential compared to the drought susceptible clones: TRFK 6/8, TRFK 12/9, TRFK 301/4, TRFK 31/11, S15/10, TRFK 7/9, TRFK 31/8 and BBK 35.

Although there were some variations in catechin levels among the individual clones in the susceptible group during dry period the differences were not statistically significant (P≤0.05). For example the average catechin content for clone TRFK 6/8 and TRFK 31/8 was much higher than that in the rest of the clones (all droughts susceptible) within the same season. The catechin levels at the end of the dry and hot period differed significantly (P≤0.05) between the drought tolerant and drought susceptible clones. The interaction between the soil moisture content and clone were also significant. Catechin content in leaves of tea remained almost constant from June-November period (that period was relatively wet, and the tea plants were not experiencing any stress) and gradually decreasing from December reaching the lowest levels towards the end of January-March season (dry and hot). Low leaf water potential resulted in increased catechin levels content in tea leaves, and there were significant correlations (P≤0.05) between soil water content and catechin content. The catechin content was consistently high in clones 303/577 and SFS 150. Ranking drought tolerance on the basis of stability suggested that clone SFS 150 was the most stable clone, followed by 303/577. The susceptible clones were, with two exceptions (6/8 and 31/8), the most unstable across the seasons.

Catechin concentrations in all the clones' increased during the dry period. These results agree with findings by Ojeda et al. (2002), Flexas and Medrano (2002) and Cheruiyot et al. (2008) who reported that, phenolic biosynthesis in tea is significantly influenced by soil moisture deficit. The results also support the findings of Esteban et al. (2001), Roby et al. (2004) and Salón et al. (2005), who reported that a direct response on phenolic biosynthesis to water deficit by a plant can be positive or negative, depending on the type of phenolic compound, the degree of water deficit and the growth period during which stress is applied.

Clones TRFK 303/577 and TRFK SFS 150 had significant levels of catechin content than the other clones in the study (Table 2). Similarly, the same clones had higher leaf water potential, indicating that they were more tolerant to water stress. Given the close correlation, these results suggest an association of catechin contents with water stress in tea. This observation agrees with results of Khan and Mukhtar (2007) who noted increased polyphenols in light and water-stress resistant safflower and cucumber seedlings as compared to

Table 2. Variation in catechin levels of ten clones over the three seasons.

Clone	Season I June-August	Season II September-November	Season III December-February
TRFK 31/8	0.4700[a]	0.4767[a]	0.2600[a]
TRFK301/4	0.4267[ab]	0.4033[ab]	0.2433[a]
TRFK311/11	0.3833[ab]	0.4033[ab]	0.2133[ab]
TRFK303/577	0.3233[bc]	0.3300[ab]c	0.2067[abc]
TRFK 6/8	0.3200[bc]	0.3167[bc]	0.1767[abcd]
TRFK 7/9	0.3100[bc]	0.3033[bc]	0.1333[bcd]
BBK35	0.2300[cd]	0.2967[bc]	0.1300[bcd]
SFS150	0.2067[cd]	0.1900[cd]	0.1200[cd]
S15/10	0.1867[d]	0.1867[cd]	0.0966[d]
TRFK 12/19	0.1800[d]	0.1300[d]	0.0900[d]

The interaction means and marginal means followed by a common letter are not significantly different at 5% levels by Duncan's multiple range tests.

those which responded weakly to the stresses.

Clones accounted for 7.5% of the treatments sum of squares while season accounted for 74.3% (data not shown). This showed a great influence that the season had on variations of catechin contents in the study. Interactions between clones and season accounted for 18.2% for mean sum of squares amongst 80% of the clones. This meant that there was substantial influence on catechin accumulation from soil water differences from one season to another.

Conclusion

The study indicate varied fluctuation of catechin content with changes in soil moisture content, and suggest that clones with more stable catechin contents across different water regimes are more tolerant to water stress. This implies that clones that have less fluctuation in catechin content are less affected by changes in soil moisture content and reflect better water stress tolerance ability.

Conflict of Interest

The authors have not declared any conflict of interest.

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REFERENCES

Borland AM, Griffiths H, Hartwell J, Smith JA (2009). Exploiting the potential of plants with crassulacean acid metabolism for bioenergy production on marginal lands. J. Exp. Bot. 60:2879-2896.

Bota J, Stasyk O, Flexas J, Medrano H (2004). Effect of water stress on partitioning of ^{14}C- labeled photosynthates in *Vitis vinifera*, *Funct.* Plant Biol. 31:697-708.

Callander BA, Woodhead T (1981). Canopy conductance of estate tea in Kenya. Agric. Met. 23:151-167.

Cherotich L, Kamunya S, Alakonya A, Msomba S, Uwimana M, Wanyoko J, Owuor P (2013). Variation in Catechin Composition of Popularly Cultivated Tea Clones in East Africa. Am. J. Plant Sci. 4:628-640.

Dale G (2006). "Epigallocatechin-3-Gallate (EGCG): Chemical and Biomedical Perspectives," Phytochem. 67(17):1849-1855.

Damayanthi MMN, Mohotti AJ, Nissanka SP (2010). Comparison of Tolerant Ability of Mature Field Grown Tea (*Camellia sinensis* L.) Cultivars Exposed to a Drought Stress in Passara Area. Trop. Agric. Res. 22(1):66-75.

Esteban MA, Villanueva MJ, Lissarrague JR (2001). Effect of irrigation on changes in the anthocyanin composition of the skin of cv Tempranillo (*Vitis vinifera* L.) grape berries during ripening. J. Sci. Food Agric. 81:409-420.

Flexas J, Medrano H (2002). Effects of drought on photosynthesis in grapevines under field conditions: an evaluation of stomatal and mesophyll limitations. Funct. Plant Biol. 29:461-471.

Kwach BO, Kamau DM, Owuor PO, Wanyoko JK, Msomba SW, Muhoza C (2011). Effects of location of production, fertilizer rates and plucking intervals on mature leaf nutrients of clone TRFK 6/8 in East Africa. Tea, 32(2):56-68.

Mayer A (2006). Polyphenol oxidases in plants and fungi: going places? A review. Phytochemistry 67:2318-2331.

Ojeda H, Andary C, Kraeva E, Carbonneau A, Deloire A (2002). Influence of pre- and postvéraison water deficit on synthesis and concentration of skin phenolic compounds during berry growth of *Vitis vinifera* cv. Shiraz. Am. J. Enol. Viti. 53:261-267.

Othieno CO, Stephens W, Carr MKV (1992). Yield variability at the Tea Research Foundation of Kenya. Agric. Met. 61:237-252.

Phene CJ, Reginato RJ, Itier B, Tanner BR (1990). Sensing irrigation needs. p. 208-261. In: G. J. Hoffman, T. A. Howell, and K. H. Solomon (eds.). Management of farm irrigation systems. ASAE., Joseph, Mich.

Roby G, Harbertson JF, Adams DO, Matthews MA (2004). Berry size and vine water deficits as factors in winegrape composition: Anthocyanins and tannins. Aust. J. Grape Wine Res. 10:100-107.

Salón JL, Chirivella C, Castel JR (2005). Response of cv. Bobal to timing of deficit irrigation in Requena, Spain: water relations, yield, and wine quality. Am. J. Enol. Viti. 56:1-8.

Watson M (1986). Soil and climatic requirements. In: Sivapalan P, Kulasegaram S, Kathiravetpillai A. (eds), Handbook on Tea, Tea Research Institute, Talawakelle, Sri Lanka. pp. 3-5.

Zobayed SM, Afreen A, Kozai T (2007). "Phytochemi-cal and Physiological Changes in the Leaves of St. John's Wort Plants under a Water Stress Condition," Environ. Exp. Bot. 59(2):109-114.

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