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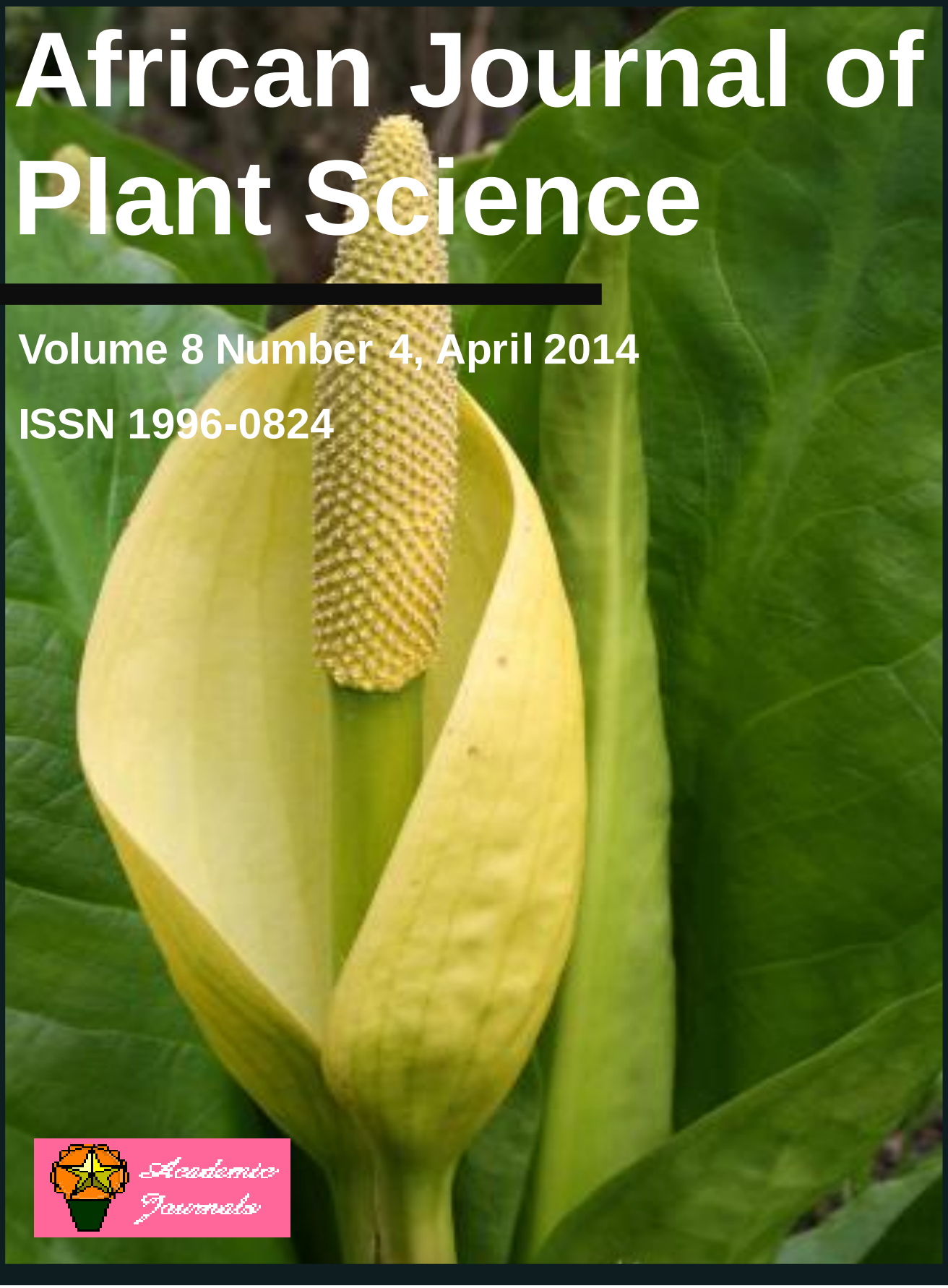

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Pitout JDD, Church DL, Gregson DB, Chow BL, McCracken M, Mulvey M, Laupland KB (2007). Molecular epidemiology of CTXM-producing Escherichia coli in the Calgary Health Region: emergence of CTX-M-15-producing isolates. Antimicrob. Agents Chemother. 51: 1281-1286.

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Full Length Research Paper

Camel urine, a potent tool for plant protection

E. Nafie

Department of Botany, College for Women (Arts, Science and Education), Ain Shams University, Cairo, Egypt.

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Cross-tolerance is the phenomenon by which a plant resistance to stress results in resistance to another form of stress. Such intriguing links has made the author to suggest that foliar camel urine (CU) application depending on its amazing constituents may mimic elicitor actions and prime tissues to pathogens in normally susceptible host plants. This study was conducted to assess anatomical and biochemical pathogenesis alterations in tomato water T1, *Fusarium oxysporum* infected T2 plants and also to evaluate the defense-related responses resulting from CU application in either nonT3 and/or T4 infected seedlings at anatomical and molecular levels. Fast and consistent increment in detoxifying enzymes peroxidase, phenylalanine ammonia-lyase, polyphenol oxidase and soluble proteins were recorded in T4 roots. In contrast, tomato roots, T2, T4 showed retard response in catalase activity. T4 accumulate highly significant level (80%) of total free and bound phenolics within its root. Root of T4 SA concomitant with coumarins had significant increment in both free and conjugated phenolic part. Anatomical microscopy shows alterations in T2, T3 as compared to T1 tomato root vascular bundle diameter. Results showed earlier reduction explained as adaptation mechanism in resisting the imposed stress regardless of its type. In contrast, T4 seedlings showed great increase in vascular bundle diameter concomitant with xylem lignin deposition and on epidermis cell walls. Tomato shoot administered CU implicate phenylpropanoid pathway in prime root to *F. oxysporum* invasion by enhancing innate immunity mechanisms, fast accumulation of H_2O_2 concomitant with cell wall modifications as physical barrier.

Key words: Cross-resistance, signaling, defense mechanism, detoxification enzymes, free and glycoside-phenolics, phenylpropanoid pathway.

INTRODUCTION

Plants are frequently exposed to different and simultaneous environmental stresses, which can be both biotic and/or abiotic. Exposure to abiotic stress, in some cases, enhances resistance to pathogens indicative of crosstalk between biotic and abiotic stress signaling (Bowler and Fluhr, 2000). Bowler and Fluhr (2000) and Abuqamar et al. (2009) stated that biotic and abiotic stress have similarities in plant responses to pathogens and abiotic stresses and partially share induced signaling cascades, among which are production of reactive

E-mail: oeetezaz@yahoo.com. Tel: +202-22736361.

oxygen species (ROS), accumulation of hormones such as salicylic acid, ethylene, jasmonic acid and abscisic acid. These signals ultimately induce expression of nuclear defense genes that lead to the assembly of the overall defense reaction.

Mittler et al. (2004) suggested that during plants evolution they were able to regulate ROS toxicity, through a highly balanced and tightly coordinated network of at least 152 genes which encode both ROS-producing and ROS-scavenging enzymes. For this reason, ROS molecules have been used as signaling molecules and accordingly, the interplay between the ROS-producing and ROS-scavenging pathways will determine the intensity, duration and localization of the ROS signals (Mittler et al., 2004).

Usually, cellular signaling cascade via ROS generated by plant-pathogen interactions, can also be activated by the use of elicitors, stable molecules that induce an immune defense response in plants (Spoel and Dong, 2012). Elicitor-induced plant signaling, serves as a guide to a series of intracellular events that end in the activation of transduction cascades and hormonal pathways, which trigger induced resistance and consequently activate plant immunity to environmental stresses (Spoel and Dong, 2012).

The exploitation of natural resources origin in conferring resistance to plants against abiotic and biotic stresses is gaining much attention. Urine and milk of camels (*Camelus dromedarius*) have been considered to have medicinal significance due to the active substances contained in desert plants (AL-awadi and AL-Jedabi, 2000). An inhibitory and antibiotic activity of camel urine against the growth of *Candida albicans* (yeast), *Aspergillus nige*r, *Fusarium oxysporum* were proven even after it's boiling to 100°C (AL-awadi and AL-Jedabi, 2000).

The urine of camel, was very much in use as remedies for the treatment of dropsy, abdominal enlargements, anaemia, abdominal tumor, tuberculosis, haemorrhoids, leucoderma, leprosy and in mental diseases (Thakur, 2004). Al-Attas (2008), using neutron activation analysis, estimated some essential and rare elements in milk and urine of camels.

In fact, it is known that treatment of plants with elicitors, or attack with pathogens, causes a set of defense reactions such as the accumulation of defensive secondary metabolites in edible and inedible parts of plants, specific gene expression and enzymatic induction (García-Mier et al., 2013).

Plants undergo also anatomical changes in response to biotic and abiotic factors. De Micco et al. (2013) conducted a study to reveal histology alterations in *Phaseolus vulgaris* L. plants when subjected to increasing dose of X-rays (0.3, 10, 50 and 100 Gy), results showed that even at high levels of radiation, general anatomical structure was not severely perturbed. There seems to be no report in the literature on the effect of CU on physiology or anatomy of higher plants. The present study suggested that CU could encourage tomato defensive mechanisms which will prime the plant against further invasion depending on its amazing constituents and its potency to mimic elicitor's role. This work aim to first, assess tomato root responses to *F. oxysporum* challenge and CU foliar application, second to test if pretreatment aerial shoot evoke root defense mechanisms against next *Fusarium* challenge. To do this, histological concomitant with biochemical changes in treated tomato seedlings different parts were assessed.

MATERIALS AND METHODS

Tomato seeds (*Lycopersicon esculentum* vs. *castlerock*) susceptible to *F. oxysporum* f. sp. *radicis- lycopersici* were kindly provided by Field Crops Research Institute at the National Research Centre, Giza, Egypt. Seeds were surface disinfected in 1% hydrochloric acid for 30 min and rinsed repeatedly in sterile double-distilled water prior to sowing (Fuchs et al., 1997), planted and grown in vermiculite in an environmentally controlled greenhouse at 24 ±2°C with 16/8 h of light - dark condition till desired age. Plants were fertilized twice a week, young plants, approximately with five or six fully expanded leaves were then transplanted into pots.

Camel urine was received from Maryout Research Station of Desert Research Center after collection from male camel of about seven years old in the early morning.

Fungal inoculums' preparation

Authentic fungal culture was received from Plant Pathology Research Institute, Agriculture Research Center, Giza, Egypt. Fungus *F. oxysporum* f. sp. *Radicis lycopersici* were sub cultured in 9-cm Petri dishes on potato dextrose agar (PDA) at 25°C in the dark. Conidia were removed from 10-15-day-old cultures into distilled water and their concentration was adjusted to 10^6 - 10^7 conidia per ml with a cytometer before inoculation.

Experimental design and treatments application

Upon the appearance of the fifth leaf, pots were randomly assigned to four groups of twenty replicates each, sprayed equally with either water (T1), and challenged with *F. oxysporum* (T2), sprayed with camel urine (T3), and challenged with *F. oxysporum* (T4). Challenging of T2 and T4 were carried out with pathogen two days later after foliar applications. The experiment was left till harvesting time: two, four, seven and fourteen days after inoculation.

Preparation of tomato tissues to study enzymatic activity

Seedlings representing all treatments at the harvesting time were separated, immediately frozen in liquid N_2 and stored at - 80°C.

Plant biochemical analysis

Phenylalanine ammonia-lyase enzyme extraction and assay (PAL) (EC 4.3.1.5)

The ratio of frozen tissue (g) and extraction buffer volume (ml) was 1:5. The extraction was carried out following the method proposed

by Lister et al. (1996). Frozen plant material was ground at 4°C in buffer (50 mM Na_2HPO_4/KH_2PO_4, pH 7, polyvinylpyrrolidone (PVP) 0, 5% (Mr, 44 000), 50 mM sodium ascorbate, 18 mM mercaptoethanol, 0.1% (v/v) Triton X-100). The homogenate was filtered through four layers of cheesecloth and centrifuged at 20 000 g for 10 min. $(NH_4)_2SO_4$ was added to the supernatant (to 35% saturation), which was then centrifuged for 20 min at 20 000 g to remove the PVP. More $(NH_4)_2SO_4$ was added to this supernatant to reach a final saturation of 80%. This fraction was centrifuged at 20 000 g for 20 min, and the pellet was re suspended in extraction buffer (without PVP and Triton). This solution was used for PAL assays.

PAL activity was assayed after adapting (McCallum and Walker, 1990) method. The assay mixture consisted of 0.06 M sodium borate buffer, pH 8.8, and partially purified enzyme. The reaction was started by the addition of 11 mM L-phenylalanine. The reaction mixture was incubated at 30°C for 15 min, and the reaction was terminated by the addition of 6 N HCl and then measured at 290 nm (Lee et al., 2007). Enzyme activity was expressed as µM cinnamic acid h^{-1} g^{-1} FW.

Peroxidase enzyme extraction and assay (POD) (EC 1.11.1.7)

Frozen plant parts were homogenized (1:5) in appropriate ice cold extraction buffer: 0.1 M potassium phosphate buffer (pH 6.8) containing 1% Triton X-100 and 0.15 g of Polyclar AT (insoluble polyvinylpolypyrrolidone, Sigma Chemical Co., St. Louis). The supernatants were used directly in the enzyme assays.

Peroxidase activity was determined following Hammerschmidt and Kuc (1982) method. The reaction mixture consisted of 0.5 ml plant extract, 1.5 ml of 0.05 M pyrogallol and 0.5 ml of 1% hydrogen peroxide. The reaction was incubated in a water bath, and then terminated after incubation for 30 min; absorbance was recorded at 420 nm. The enzyme activity was expressed as change in the absorbance of the reaction mixture, min^{-1} g^{-1} FW.

Catalase enzyme extraction and assay (CAT) (EC 1.11.1.6)

Frozen plant parts stored at - 80°C, were extracted in 0.4 ml of buffer (potassium phosphate, 50 mM at pH 7.4, containing 10 mM DTT). Catalase activity was assayed at 25°C following the decrease in absorption at 240 nm in 10 mM sodium phosphate buffer, pH 7(Aebi, 1984). The enzyme activity was expressed as mM H_2O_2, min^{-1} g^{-1} FW.

Polyphenol oxidase enzyme extraction and assay (PPO) (EC 1.14.18.1)

The extraction method proposed by Thypyapong et al. (1995) after some modifications were used. Frozen tissue was grounded in extraction buffer (100 mM Tris-HCl, pH 7.0, 100 mM KCl, 1 mM phenylmethanesulfonyl fluoride (PMSF) and 3% [w/v] PVP). The homogenates were centrifuged at 12 000g for 15 min, and the supernatant was used to measure the PPO enzyme activity. The PPO activity was determined according to Mayer (1995) with some modifications. The reaction mixture consisted of 100 mM buffer (Na_2HPO_4/ KH_2PO_4), pH 7.0 and 1.5 ml of 0.01 M catechol and reaction was initiated by the addition of 200 µl enzyme extract, the activity was expressed as changes in absorbance at 495 nm.

Protein determination

From each treatment, 0.5 g samples were grinded in mortar with 5 ml of phosphate buffer pH 7.6 and centrifuged at 8000 g for 20 min. The supernatant was then made equal by adding phosphate buffer solution. Coomassie Brillaint Blue G-250 solution was then added and mixed thoroughly (Bradford, 1976). The total volume was 3 ml. All treatments were incubated for 5 min at room temperature and absorbance at 595 nm was recorded against the reagent blank. A standard sample (100 µg in 100 µl) was used to estimate soluble protein concentrations in the unknown treated samples as mg g^{-1}.

Phenolics extraction

Tomato seedlings harvested from two, four, seven and fourteen days old plants, representing each of the four treatments were used to extract a free-phenolic fraction part, and a conjugated fraction consisting of the aglycones released after hydrolysis. The extraction method of Fawe et al. (1998) was used. Fine powder samples preserved in liquid N_2 were extracted in 80% acidified methanol (10 g/100 ml). The extract was then filtered with glass fiber filters (GF/C; Schlei and Schuell), and the filtrate was concentrated. The aqueous residue was adjusted to pH 2.0 and partitioned against hexane. The aqueous phase containing the phenolic constituents was further partitioned against ethyl acetate and then subjected to acid hydrolysis, 4 N HCl in an autoclave for 15 min. The hydrolysate was cooled and partitioned against ethyl acetate. The two ethyl acetate fractions obtained were dried, and the residues designated as free-phenolic fraction and conjugated-phenolic fraction, respectively, were re suspended in absolute methanol (2.5 g/ml).

Total phenolic concentration determination

Total phenolic (TP) concentration was measured in both free and glycosidic-bound phenolic extract by using Folin Ciocalteus reagent (Sigma, St. Louis, Mo.) according to Singleton and Rossi (1965) method. Determination of total phenols concentration was done using a Jenway (UV/Vis 6405) spectrophotometer monitoring 750 nm. TP content was expressed as mg FW.

Phytoalexins separation using high-performance liquid chromatography (HPLC)

Tomato leaves and roots from all treated plants after 48 h of infection were harvested, pooled and kept at -80°C until use (approximately 20 g). The phenolic compounds extraction was performed as described previously. The resultant free and conjugated-phenolic fractions after separation as previously described were used to determine their profiles.

The HPLC system (Thermo Separation Products, Riviera Beach, Fla.) consisted of an auto sampler (AS3000), injector (100 µl), column oven (30), pump (P3000) and diode array detector (UV6000). A reverse-phase C18 column (250 by 4.6 mm; Luna 2; Phenomenex, Torrance, Calif.), with a pre column of similar resin, was employed. Elution was performed using phosphate buffer (50 mM, pH 2.5) and methanol at a flow rate of 1 ml min^{-1}. A linear-gradient program was developed as follows: C18 column (time [in minutes]/methanol [percent]) = 0/0, 6/0, 31/95, 32/0. In all instances, the software was programmed to show peaks at their maximum absorbance. For each treatment, HPLC analyses were repeated twice, showing similar results.

Tissue processing for ultra structural investigation

For optical light microscopy (OLM) investigation, roots (covering 7 and 14 day harvesting time) sections from chosen tomato plant parts were fixed in FAA (ethanol 50% + formaldehyde 5% + glacial acetic acid 10%, in water) for 48 h. After fixation, segments were

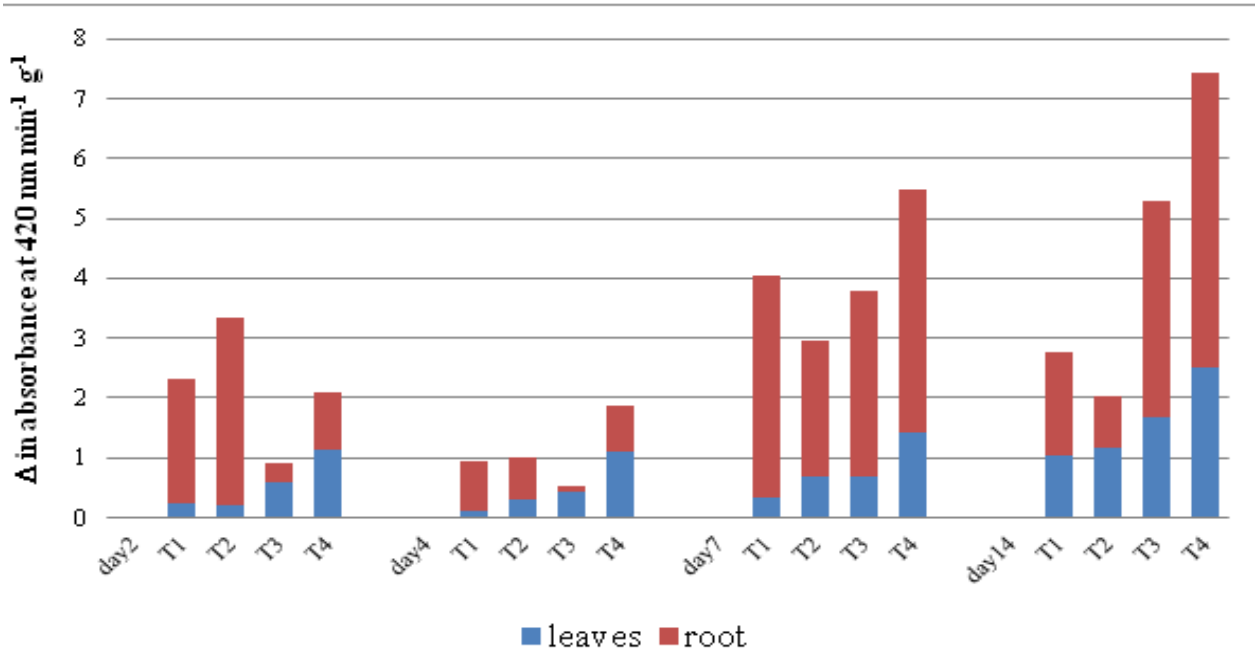

Figure 1. Peroxidase enzyme activity estimated in water (T1), and challenged with *F. oxysporum* (T2), sprayed with camel urine (T3), and challenged with *F. oxysporum* (T4), harvested at day two, four, seven and 14 post infection.

dehydrated through a gradient series of ethanol, cleared with xylene, and finally embedded in paraffin. Fifteen-micrometer-thick sections were cut with a microtome and transferred onto glass slides coated with egg albumin. Sections were de-paraffinized with xylene and rehydrated through a gradient series of ethanol. Sections were stained with safranin O (at ethanol 50% step) to show lignin following Balatinecz and Kennedy (1967) with slight modifications, washed well, then light green was used (at ethanol 100% step) for visualizing the plant sections anatomy. Photographs were taken using Olympus microscope BH2 with camera attachment (model U-ACAD-2). Calibrated linear ocular was used for tissue measurements.

Statistical analysis

The test of least significant difference (L.S.D) at the level of 0.05% significance was used to examine differences among treatment means and interactions. Data were statistically analyzed using MSTAT-C software package according to the described method by Freed et al. (1989).

RESULTS

Biochemical changes

Camel urine application enhanced tomato resistance responses

To assess pathogenesis and if pre CU application may prime and condition plant response to successive pathogen attack, changes in detoxification enzymes were elucidated in samples harvested at the required time.

Peroxidase and catalase activities

The fungus inoculation resulted in rapid increment in T2 root enzyme activity after 48 h thereafter fluctuated, while leaves exhibited progressive levels. Peroxidase activity in T4 treated plants showed progressive local and systemic enhancement *in vivo* plant leaf and root. Root displayed higher activity than leaves as shown in Figure 1. Leaves of infected tomatoes exhibited CAT increment two days later while their roots negatively responded. T4 leaves during same time showed similarity with the control while roots enzyme sharply decreased. At the 14th day, enzyme activity in roots of all treated plants were increased, on the other hand leaves responded in a different manner as shown in Figure 2.

Alterations in phenolic compounds metabolism

Phenylalanine ammonia-lyase and polyphenol oxidase activities

Phenylalanine ammonia-lyase and polyphenol oxidase activities in T4 roots progressively increased till 14 day post challenge. Roots of T2 treated plants had highest level after 24 h in PAL and PPO activity thereafter

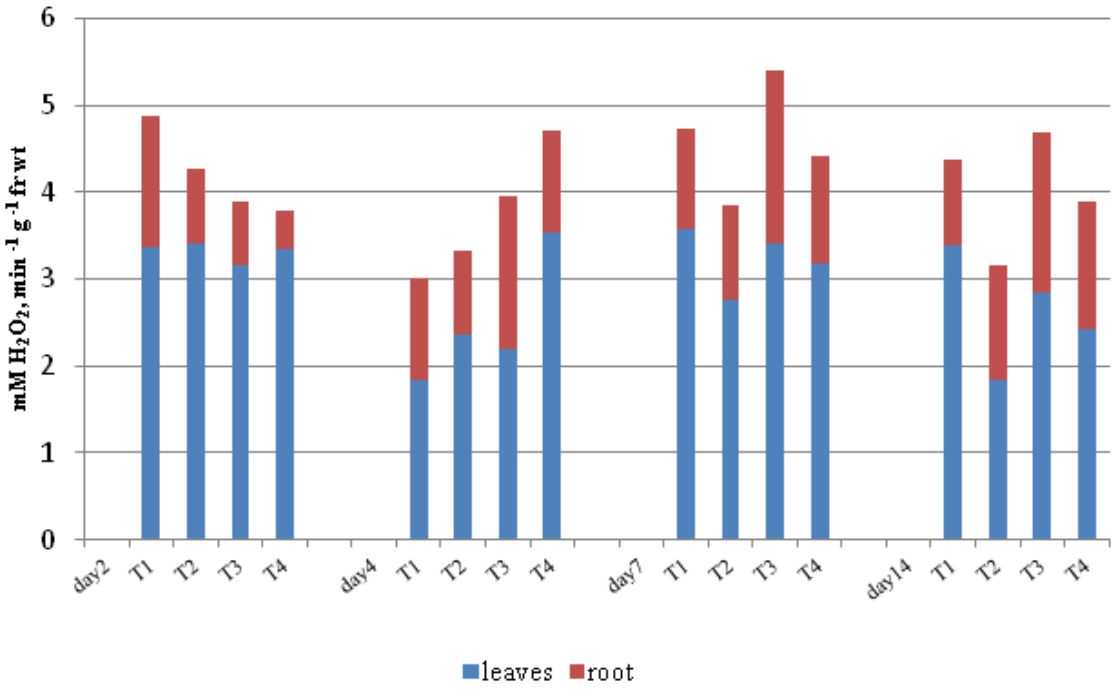

Figure 2. Catalase enzyme activity estimated in water (T1), and challenged with *F. oxysporum* (T2), sprayed with camel urine (T3), and challenged with *F. oxysporum* (T4)l, harvested at at day two ,four, seven and 14 post infection.

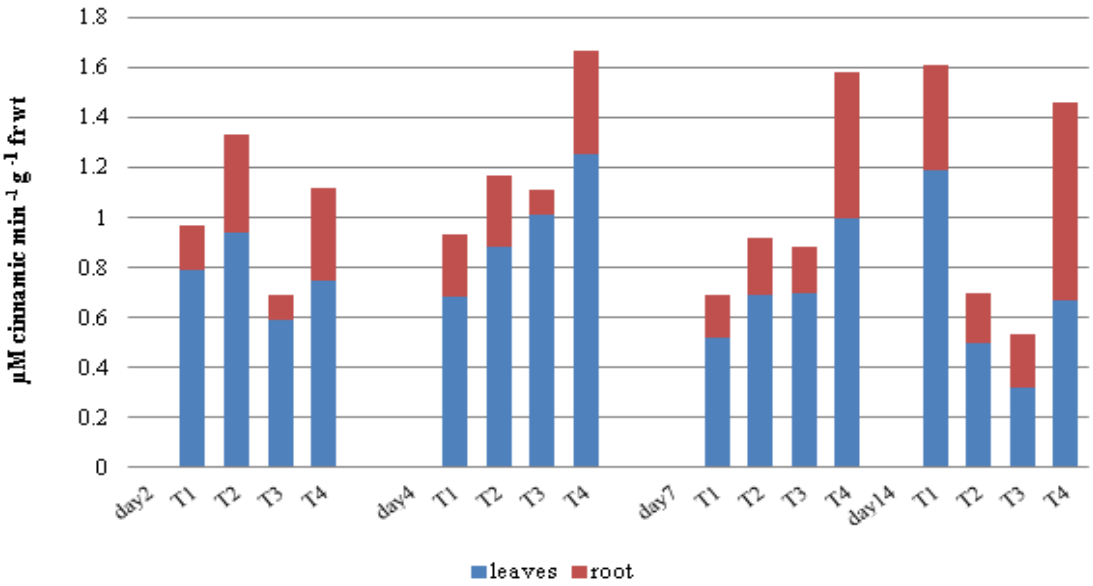

Figure 3. Phenylalanine ammonia-lyase enzyme activity estimated in water (T1), and challenged with *F. oxysporum* (T2), sprayed with camel urine (T3), and challenged with *F. oxysporum* (T4), harvested at day two, four, seven and 14 post infection.

generally decreased as compared to the control. Fungus inoculation caused rapid increment in leaves PAL activities then gradually ceased while PPO showed fluctuations as shown in Figures 3 and 4.

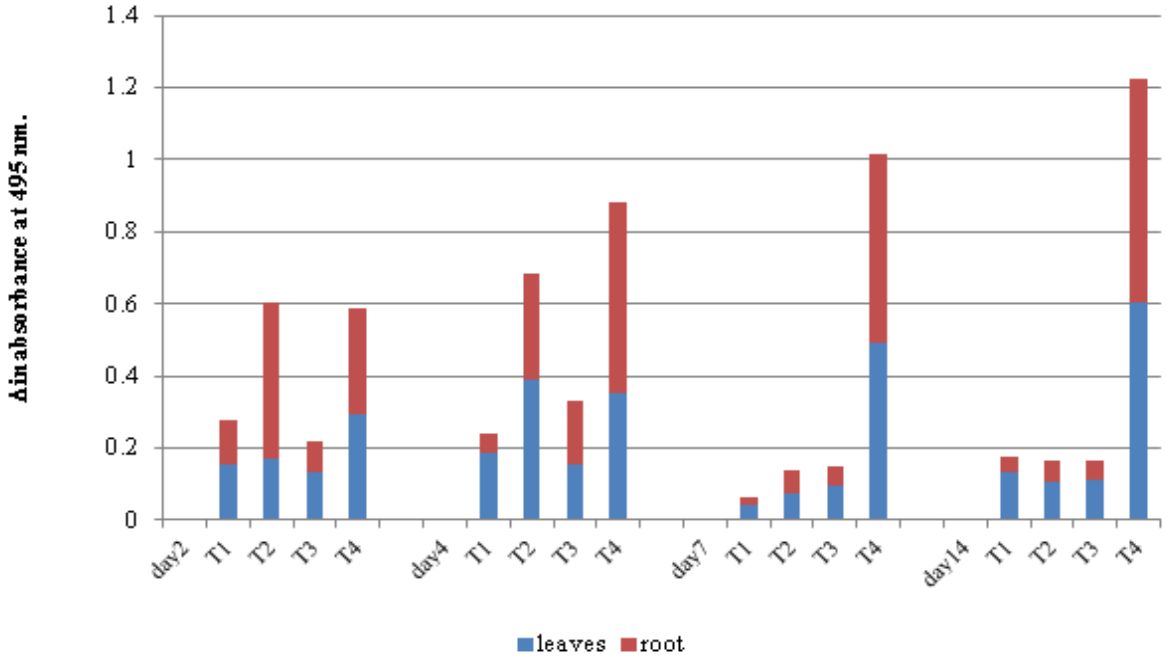

Figure 4. Polyphenol oxidase enzyme activity estimated in water (T1), and challenged with *F. oxysporum (*T2), sprayed with camel urine (T3), and challenged with *F. oxysporum* (T4), harvested at two day two, four, seven and 14 post infection.

Quantification of total phenolics in free and conjugated phenolic extracts

Increment in total phenols throughout the study was recorded in both free and conjugated phenols in different tomato parts as shown in Table 1. Alterations in phenolic compounds recorded throughout the experiment were plant part dependent, in which T4 root recorded the highest level. T2 and T4 TP in tomato leaves showed increment reaching 43.8 and 29% respectively, and in root, 40 and 81.7% at the 14 day post infection with respect to each control.

HPLC separation of phytoalexins

A comparative study for 48 h post harvesting of plants leaves and roots represent all treatments profile of certain fractions analyzed in conjugated and free phenolics, using reverse-phase HPLC. Four phenolic compounds were observed, and changes in their relative amount distribution between conjugate and free state were recorded as shown in Table 2.

Leaves and roots of challenged tomato plant with *F. oxysporum* T2 showed general retarded levels in free and conjugated coumarins, pyrogallol, catechol and salicylic acid amounts relative to the control T1 that was not challenged. In T4 tomato roots, coumarins, and salicylic acid were enhanced significantly in free and glycosidic-bound phenolic fraction while leaves salicylic acid enhanced bound phenolic fraction.

Soluble proteins

Soluble proteins in T2 or T4 roots showed continuous increment over the control. In T2 and T4 seedlings leaves rapid and negative response was recorded and continued to increase in T4 only, results are illustrated in Figure 5.

Histological changes

A part of root transverse sections from all treated tomato seedlings seven and fourteen days post-harvest are in Table 3 and Figures 6 and 7 to explore histological changes.

Tomato root section showed abundance in xylem ray with large prominent metaxylem vessels in early root as shown in Figure 6a. The structural changes associated with pathogen infection in T2 tomato seedlings root cortex and vascular bundle was reduction in both diameters, as compared to the control, T1 (Figure 6). In seedling root T3, cortex diameter was larger while vascular bundle was reduced as compared to the control T1 (Figure 6). Infection after foliar pretreatment of seedlings T4 enhanced lateral root initiation, xylem cell wall lignifications in the form of dark lesions encircling the cells as shown in Figure 6 and increment is not only in

Table 1. Phenolic compounds (free and conjugate) estimated in tomato leaves and roots in water (T1), challenged with *F. oxysporum* (T2), sprayed with camel urine (T3) and challenged with *F. oxysporum* (T4) harvested at two, four, 7 and 14 day post infection.

Harvesting days		2 day			4 day			7 day			14 day			
Phenolic (mg/g FW) treatments		Free	Glycosidic-bound	Total	Free	Glycosidic-bound	Total	Free	Glycosidic-bound	Total	Free	Glycosidic-bound	Total	Total (%)
Leaves	T1	0.4c	0.52b	0.92d	0.73b	0.85b	1.58c	0.8c	0.90d	1.7d	0.92c	1.20c	2.12d	
	T2	0.75b	0.60b	1.35c	1.32a	1.11b	2.43b	1.46a	1.22c	2.68c	1.66ab	1.39c	3.05c	43.80
	T3	0.68b	1.43a	2.11b	1.2a	2.20a	3.40a	1.27b	2.51b	3.78b	1.50b	2.65b	4.15b	
	T4	1.07a	1.46a	2.53a	1.4a	2.44a	3.84a	1.45a	2.90a	4.35a	1.85a	3.52a	5.37a	29.30
	LSD at 5%	0.21	0.45	0.32	0.27	0.48	0.63	0.30	0.26	0.64	0.28	0.65	0.73	
Roots	T1	0.69c	0.94b	1.63bc	0.95c	1.14c	2.09c	0.98 c	1.40b	2.38 c	1.00 c	1.50c	2.50c	
	T2	1.00b	0.92b	1.92b	1.48b	1.34c	2.82b	1.61 b	1.53b	3.14 b	1.80 b	1.70c	3.50b	40
	T3	0.55c	0.99b	1.54c	0.57d	1.60b	2.17c	0.75 c	1.78b	2.53 c	1.86 b	2.35b	4.21 b	
	T4	1.56a	2.45a	4.01a	1.87a	3.67a	5.54a	2.60 a	4.41a	7.01 a	2.74 a	4.91a	7.65 a	81.70
	LSD at 5%	0.26	0.35	0.23	0.31	0.22	0.54	0.48	1.44	0.50	0.68	0.57	0.85	

Table 2. Certain phenolic compounds estimated using HPLC (free and glycoside) in leaves and roots extracted after forty-eight hours (after infection) in either water (T1), and challenged with *F. oxysporum* (T2), sprayed with camel urine (T3), and challenged with *F. oxysporum* (T4). Phenolics estimated as µg/g FW.

Parameter		Free phenolics				Glycosidic –bound				Total			
		Coumarins	Pyrogallol	catechol	salicylic acid	coumarins	pyrogallol	catechol	salicylic acid	coumarins	pyrogallol	catechol	Salicylic acid
Leaves	T1	101.63a	50.10b	65.25a	13.9b	217.36a	101.6a	114.21a	7.95c	318.99a	151.70a	179.46a	21.85a
	T2	58.62b	59.00a	60.85a	6.4bb	68.50b	27.5c	36.60b	2.10d	127.12b	86.50b	97.45b	8.50d
	T3	32.12c	21.00c	26.36b	1.9c	66.74b	72.52b	14.35c	13.22a	98.86c	93.52b	40.71c	15.12b
	T4	33.74c	17.13c	22.00b	2.1c	32.50c	14.31d	12.64c	9.62b	66.24d	31.44c	34.64c	11.72c
	LSD at 5%	5.32	4.85	8.67	4.62	10.38	9.88	11.05	1.26	13.22	10.58	15.35	2.87
Roots	T1	40.76b	35.54b	32.50a	4.27c	4.50c	10.20c	29.18b	1.75c	45.26c	45.74b	61.68b	6.02c
	T2	-	30.90b	14.63c	0.30d	6.31c	9.70c	24.44c	4.23c	6.31d	40.60bc	39.07d	4.53c
	T3	38.62b	50.00a	36.00a	8.44b	59.68b	39.63a	40.00a	11.00b	98.3b	89.63a	76.00a	19.44b
	T4	78.00a	10.60c	27.52b	10.66a	65.33a	23.45b	23.62c	17.10a	143.33a	34.05c	51.14c	27.76a
	LSD at 5%	10.24	8.65	4.32	2.08	4.65	5.34	3.98	4.21	9.25	8.62	7.36	5.14

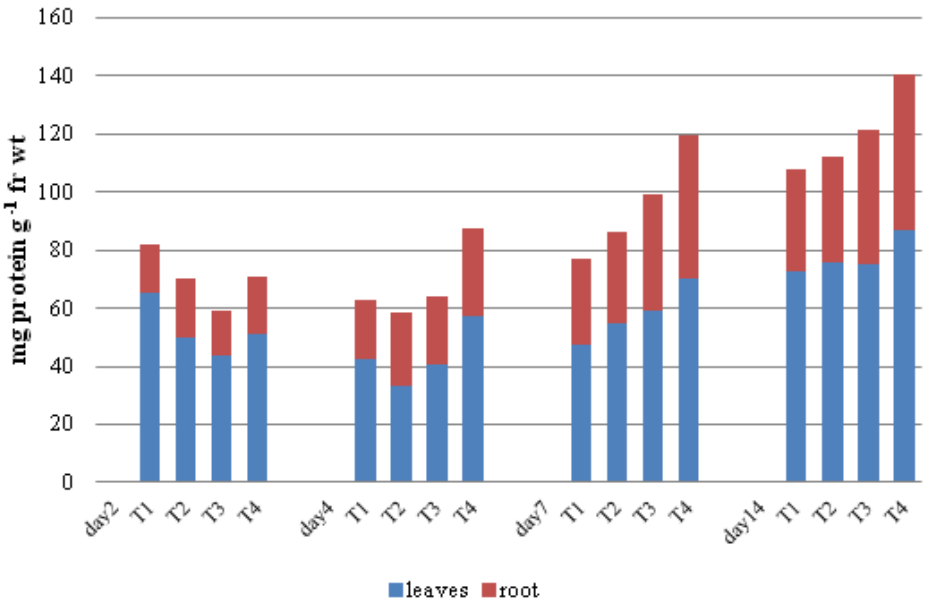

Figure 5. Soluble proteins estimated in water (T1), and challenged with *F. oxysporum* (T2), sprayed with camel urine (T3), and challenged with *F. oxysporum* (T4), harvested at day two, four, seven and 14 post infection.

Table 3. Tomato seedlings root, cortex and stele cross section diameter (µm) in either water (T1), and challenged with *F. oxysporum* (T2), sprayed with camel urine (T3), and challenged with *F. oxysporum* (T4), harvested at 7 and 14 days post infection.

Treatments	Root		Cortex		Stele	
	7 day	14 day	7 day	14day	7 day	14 day
T1	21.6	26.0ab	4.0b	5.0b	12.0b	13.0b
T2	20.0	24.0b	3.4b	4.8b	11.4b	12.6b
T3	21.0	24.60.b	5.0a	6.0a	11.0b	12.4b
T4	23.6	27.8a	3.4b	5.0b	14.4a	16.4a
LSD at 5%	NS	2.44	0.85	0.79	0.2.10	0.57

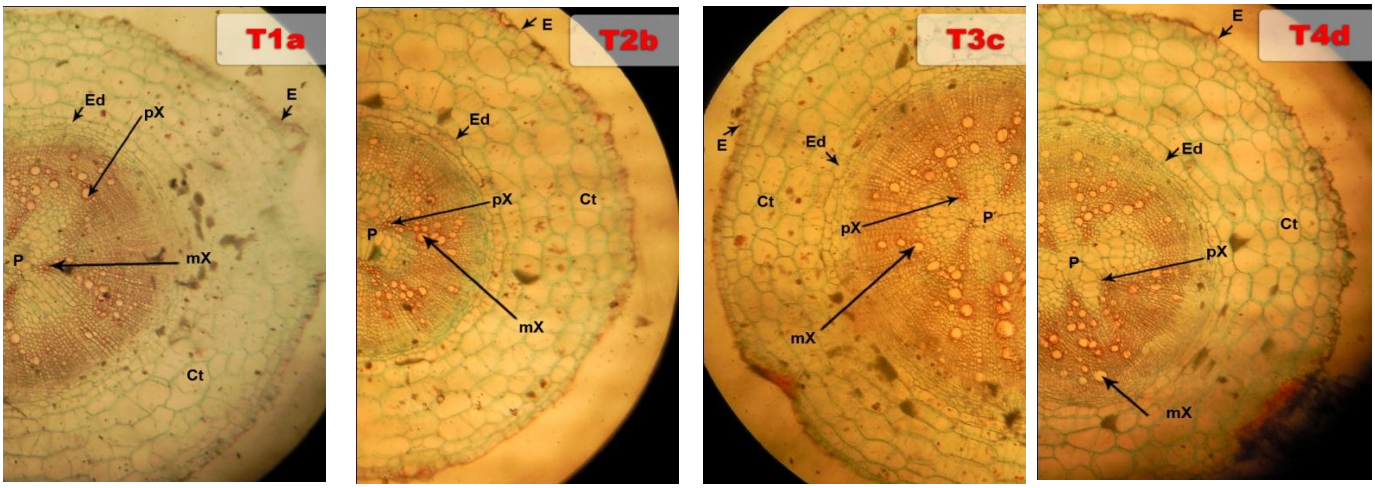

Figure 6. Light Microscopic images showing cross sections of root from 7-day post infection tomato seedlings. Different treatments were: T1a, control water non infected; T2b, *F. oxysporum* infected; T3c, urine camel foliar spraying; T4d, urine camel sprayed and infected. Ct, tomato seedlings cortex; E, epidermis; Ed, endodermis; mX, metaxylem; P, pith; Ph, phloem; pX, protoxylem.

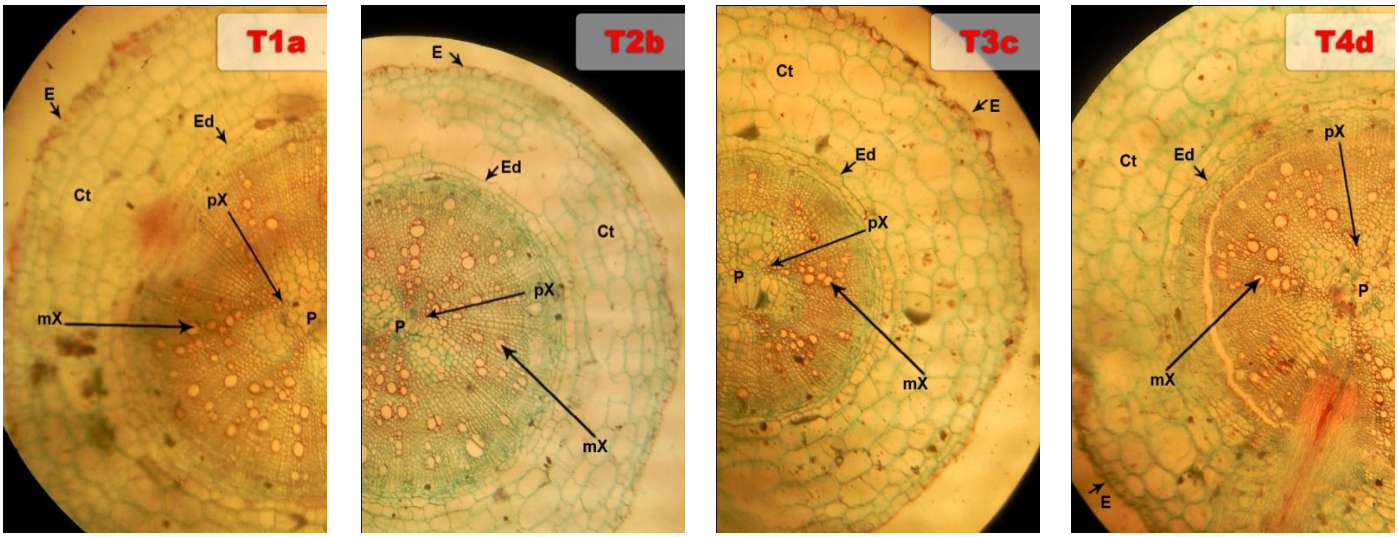

Figure 7. Light Microscopic images showing cross sections of root from 14-days post infection tomato seedlings. Different treatments were: T1a, control water non infected; T2b, *F. oxysporum* infected; T3c, urine camel foliar spraying; T4d, urine camel sprayed and infected. Ct, tomato seedlings cortex; E, epidermis; Ed, endodermis; mX, metaxylem; P, pith; Ph, phloem; pX, protoxylem.

xylem cell width but also in number. Root age follow the same trends (Figure 7).

DISCUSSION

In nature, plants often deal with simultaneous or subsequent abiotic and biotic stresses which can influence the primary induced defense responses (Abuqamar, 2009). Activation of plant defense mechanisms is associated with ecological fitness costs. Hence, plants need regulatory mechanisms to effectively and efficiently adapt to changes in their complex environment. Share between different signaling pathways provides the plant with such a powerful regulatory potential (Abuqamar, 2009).

Presented results show that tomato plants responded to exogenous, foliar applications of the CU solution. The amazing solution was found to sensitize susceptible tomato plants to react more rapidly and more efficiently to *F. oxysporum* f. sp. *Radicis lycopersici* attack.

Peroxidase has often been used as an enzymatic marker in studies of defense-related processes (Karthikeyan et al., 2006). The enhanced peroxidase activity shown by the *pschit*, transgenic tobacco lines, was linked to their increased pathogen resistance (Dana et al., 2006). Tomato tissues responded to CU application and this point to CU recognition. Tomato leaves tissues point to fast and consistent POD increments thereafter CU systemic transporting, preferentially enhanced the roots peroxidase. In seedlings roots, peroxidase activity increments inT4 implicate wall-bound phenolics accumulation in lignifications with pathogen restriction in systemic parts as a result of pre CU foliar application (Karthikeyan et al., 2006). Root CAT depletion (in the present work) may be explained by salicylic acid binding affinity to iron-containing enzymes (Klessig et al., 2000) resulting in H_2O_2 accumulation to directly award off pathogen spreading.

PAL is a key enzyme in the biosynthesis of phenyl propane unit. Tomato plant pretreated by camel urine as compared to water spray before challenging both implicated total phenolics and PAL activity increment in roots within the plant strategy to lignin deposition which had been confirmed by the histological part resulted in physical protection enhancement. Increased PAL activity level in response to pathogen or elicitor like raw cow's milk spray has been reported (Arun et al., 2010).

Karthikeyan et al. (2006) stated that infected chickpea and palm seedlings treated with biocontrol agents resulted in increased accumulation of phenolics and PAL. Tomato T2 roots suggested that immediate transient activation in PAL after infection could re-establish the notion that plant recognize its enemy and respond to invasion by PAL expression.

PPOs are a group of copper containing enzymes that catalyze oxidation of hydroxy phenols to their quinone derivatives, which have antimicrobial activity (Thipyapong and Stiffens, 1997). Recently, study on elicitation of resistance and defense related enzymes by amino acids and raw cow's milk (RCM) in Pearl millet against downy mildew disease (Arun et al., 2010) recorded increased PPO in both RCM and amino acids treated plants.

The prominent increase in PPO roots of T4 treatments exhibited the highest enzyme levels, involved camel urine spray in conditioning root defense responses against

pathogen toxic metabolites which may activate phenol-oxidizing enzymes where *F. oxysporum* was found to produce such a toxic metabolite.

Emerging evidence suggests that hormones signaling pathways, as well as ROS, play key roles in the crosstalk between biotic and abiotic stress signaling (Abuqamar, 2009). They act synergistically or antagonistically with each others to regulate plant responses to pathogens and abiotic stress factors (Abuqamar, 2009). SA quantity in roots of T4 treated seedlings, showed that SA could be a signaling molecule forming a feedback amplification cycle in concert with ROS (Abuqamar, 2009). In this way, SA induction is not required but the endogenous SA present amplifies the effects of ROS initial levels. This study contributes to defining the role of SA during the camel urine application. Asselbergh et al. (2007) found that in tomato, increase in SA-regulated defense gene expression, faster accumulation of H_2O_2 and the associated cell wall modifications explain the increase in resistance to *Botrytis cinerea*. In the present work, root of the pretreated seedlings with camel urine and pathogen challenged record enhancement conjugated SA concomitant with catalase inhibition and peroxidase increment so, shoot perception to camel urine and the cascade signaling pathways cross-talk *via* specific systematic signals like SA to prime root to other stress by enhanced defense gene expression, fast accumulation of H_2O_2 concomitant with cell wall modifications as physical barrier after lignin deposition. Cvikrová et al. (2006) conducted a study to identify the defense-related alterations in phenylpropanoid pathway, they explain the rise of phenolic contents in the reaction zones by scoring predominately derivatives of cinnamic acid, in particular *p*-coumaric acid, content of which increased by about 2.3- and 3.0-fold.

Many of the pathogen-induced phenylpropanoids like coumarins are considered phytoalexins because they exhibit antimicrobial properties (Cvikrová et al., 2006). Immediate excellent increment after two days post infection in total coumarins in T4 seedlings roots share increments in total antioxidant defenses. Further physiological and molecular research has to be done in order to clearly define the signaling pathways involved in camel urine induced resistance. Current accumulation of proteins in CU treated tomato may provide a storage form of nitrogen that is re-utilized when stress is over, while increasing soluble proteins in infected and pretreated may be due to synthesis structural proteins in particular synthesis of those proteins which are involved in modification of cell wall especially in roots to collaborate defense against pathogen infection.

In the present work, root of seedlings received both foliar application and pathogen challenged showed no tissue injury or disorganization in vascular tissues. Enhanced lignifications on epidermis cell wall and enhanced thickness inside xylem cell wall which were correlated with peroxidase activity and related to camel urine unique constituents especially Ca and Se ions as compared to water treated seedling. Selenium ions as one of CU constituents (Al-Atta, 2008) has been shown to protect plants from both herbivore and pathogen infection (Hanson et al., 2004).

In the light of the above recorded results, the present study may suggest that tomato aerial parts can recognize and respond to CU, evoke cascade signaling pathways, systematically crosstalk with roots using SA and sensitize tomato underground parts to respond faster and to a greater extent to *F. oxysporum* attack. A better understanding of these mechanisms will make it possible to genetically engineer crops disease resistance, leading to greater food production.

Conflict of Interests

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

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Full Length Research Paper

Effect of growing media on seed germination and seedling growth of papaya cv. 'Red lady'

R. L. Bhardwaj

Krishi Vigyan Kendra, Sirohi, Agriculture University Jodhpur-342304, Rajasthan, India.

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This study was carried out to explore the effect of growing media on seed germination and seedling growth of papaya (*Carrica papaya*). The research was conducted at the model nursery of Krishi Vigyan Kendra, Sirohi during two successive seasons from July to August 2008-2009 and 2009-2010. This research was arranged in a complete randomized design with nine treatment combinations and three replications. The treatments were combination between types of media with level of cocopeat. Each treatment has 100 poly bags. The results showed that the medium of vermicompost + sand + pond soil (1:1:1) with 2 cm cocopeat in top of the poly bags (T_9) gave maximum speed of emergence (277.6 and 709.09), highest germination percent (95.27 and 90.15%), highest seed vigour (91.97 and 86.69), maximum germination index (7.15 and 7.22), germination value (17.33 and 33.83) and least time required for imbibition (9.45 and 9.30 days) and minimum germination period (3.70 and 2.75 days), respectively in both years of experimentation. This medium was also found to be the best medium for the growth of papaya seedlings as it gave the highest parameters in term of seedling height (23.43 and 22.67 cm), leaf area (349.33 and 329.20 cm^2), number of leaves (10.02 and 9.67), stem girth (3.16 and 3.48 mm), number of roots (17.20 and 16.17), root length (10.20 and 9.67 cm), production of total biomass (5.02 and 4.77 g/plant) and least root/shoot ratio (0.22 and 0.20). This treatment also significantly reduces the seedling mortality and produce maximum healthy seedlings (92.23 and 93.15%) in minimum days (35.33 and 35.15) with highest net profit (Rs. 3493.30/1000 seedling and Rs. 3448.00/ 1000 seedling) and B:C ratio (1.85 and 1.84) of seedlings, in both years (2008-09 and 2009-10), respectively.

Key words: Seedling, plant growth, cocopeat, pond soil, vermicompost, B:C ratio.

INTRODUCTION

Use of suitable growing media or substrates is essential for production of quality horticultural crops. It directly affects the development and later maintenance of the extensive functional rooting system. A good growing medium would provides sufficient anchorage or support to the plant, serves as reservoir for nutrients and water, allow oxygen diffusion to the roots and permit gaseous exchange between the roots and atmosphere outside the root substrate (Abad et al., 2002). Nursery potting media influence quality of seedlings produced (Agbo and

E-mail: rajubhardwaj3@gmail.com

Omaliko, 2006). The quality of seedling obtained from a nursery influences re-establishment in the field and the eventual productivity of an orchard (Baiyeri, 2006). Papaya is an important fruit crop which is propagated by seeds only. Seed germination is affected by many factors, which include type of substrate used, environmental factors such as oxygen, water, temperature and for some plant species, light (Hartmann et al., 2001). The germination of seed of papaya (*Carrica papaya*) is frequently reported to be slow, erratic and is incomplete (Chako and Lange, 1966). Red lady is choicest variety of papaya grower due to hermaphrodite nature and prolonged self life of fruits. But the seed cost of this variety is very high (Rs. 2.0 lakh kg^{-1}) so, increasing germination percent and producing more healthy seedling is a challenge for papaya growers. The germination of papaya (*C. papaya*) cv. Red lady seeds faces certain problems in germination and has high seedling mortality due to damping off disease in nursery stage. Initial mortality and incomplete germination is also one of the causes of reduced survival percent of papaya plants. In heavy soil without enough drainage, the development of root system is suppressed and plants are more susceptible to soil borne diseases (Beattie and White, 1992). The papaya seed is endosed within a gelatinous sarcotesta (aril or outer seed coat which is formed from the outer integument). Growing media also plays important role for seed germination. Growing medium not only acts as a growing place but also as a source of nutrient for plant growth. Media composition used influences the quality of seedling (Wilson et al., 2001). Generally, media for fruit crop seedling are composed of soil, organic matter, pond soil and sand. The pond soil is usually used as a basic medium because it is cheapest and easy to procure. Supplementing of the sand is aimed to make media more porous while the organic matter (FYM and vermicompost) is added so as to enrich adequate nutrients for the seedling. There is better relationship between the manure and rooting rather than conventional soil mix and less susceptibility of the seedling to soil borne pests and diseases (Akanbi et al., 2002). Several studies on growth media had been conducted on the various fruit commodities by previous researchers. The best growth of mangosteen seedling was reached on soil medium as compared to the other media (Jawal et al., 1998). Baiyeri (2003) mentioned that the best seedling qualities of African breadfruit (*Treculia africana* Decne) were obtained when grown in medium formulated with top soil + poultry manure + river sand in 1:2:3 (v/v/v) ratios. Humic acids (vermicompost) applied in the medium increased plant height, leaf area and dry weight of peppers, tomatoes and marigold (Arancon et al., 2004). Ratna et al. (2006) working on banana cv. Raja Serai proved that soil and sand medium was the most suitable medium for shoot and leaf growth of this banana. *Uapaca kirkiana* Müell Arg. planted on the medium comprising 75% forest soil and 25% sawdust produced the tallest seedlings, larger root collar diameter and higher survival at 10 months after planting (Mhango et al., 2008).

Cocopeat is an agricultural by-product obtained after the extraction of fiber from the coconut husk (Abad et al., 2002). As a growing medium, cocopeat can be used to produce a number of crop species with acceptable quality in the tropics (Yahya and Mohklas, 1999; Yau and Murphy, 2000). Cocopeat is considered as a good growing media component with acceptable pH, electrical conductivity and other chemical attributes (Abad et al., 2002). Cocopeat has good physical properties, high total pore space, high water content, low shrinkage, low bulk density and slow biodegradation (Evans et al., 1996; Prasad, 1997). The results of many experiments revealed that cocopeat used alone, or as a component of soil medium, is suitable for roses (Blom 1999), gerbera (Labeke and Dambre, 1998), many potted plants (De Kreij and Leeuven, 2001; Treder and Nowak, 2002) and also for vegetables. Due to usually high initial level of potassium and sodium in cocopeat, the fertilization program should be adjusted carefully to plant requirements.

Keeping in view the influence of media in germination and seedling growth of papaya, the present investigation was carried out to study the effect of different media viz. sand, pond soil, FYM, vermicompost and cocopeat on seed germination, seedling growth and vigour of papaya seedlings.

MATERIALS AND METHODS

Seed material and treatment

Seed germination and seedling growth experiments of papaya were carried out at NHM Model nursery of Krishi Vigyan Kendra-Sirohi (Rajasthan) during two successive season from July to August, 2008-09 and 2009-10. Experimental treatments comprised of nine treatment combinations consisting of different combination of growth media and cocopeat filling at the top of seedling poly bags (10 x 12 cm) namely, T_1 – Sand + pond soil (1:1) without cocopeat, T_2- Sand + pond soil (1:1) with 1 cm cocopeat, T_3- Sand + pond soil (1:1) with 2 cm cocopeat, T_4- FYM + Sand + pond soil (1:1:1) without cocopeat, T_5- FYM + Sand + pond soil (1:1:1) with 1 cm cocopeat, T_6- FYM + Sand + pond soil (1:1:1) with 2 cm cocopeat, T_7- Vermicompost + sand + pond soil (1:1:1) without cocopeat, T_8- Vermicompost + sand + pond soil (1:1:1) with 1 cm cocopeat, T_9- Vermicompost + sand + pond soil (1:1:1) with 2 cm cocopeat. The seed sowing was done in month of July about 1 cm deep in different media as per treatments. The poly bags were irrigated immediately after seed sowing and repeated every day till the final emergence. After the completion of germination, the bags were irrigated once in 2 days.

Experimental design and measured parameters

For seed germination and seedling growth experiments, treatments of the experiment were conducted in complete randomized design with three replications. Each treatment was composed of 100

polybags seedlings. All the observation on germination parameters were recorded at the time of germination and growth parameter at the time of transplanting (45 days after seed sowing) from 100 seeds for germination parameter and randomly selected 10 seedling for growth parameters. Data on germination was recorded from the first germination until no further germination at two days interval. The imbibition period, number of days from sowing to commencement of germination, was recorded for all studied treatments. The rate of emergence (RE) was calculated according to Islam et al. (2009) using the following formula:

$$\text{Rate of emergence} = \frac{\text{No. of seedlings emerged 5 days after sowing}}{\text{No. of seedlings emerged 15 days after sowing}} \times 100$$

The germination percentage was calculated as the percent of germinating seeds starting from the first germination to no further germination. Germination percentage was calculated by number of germinated seedling divided by the total number of seeds sown in poly bags and multiplied by 100. The germination period was calculated as the difference between initial and final emergence (number of days) recorded. Seed vigour was calculated by total number of healthy seedling divided by the number of total seedlings and multiplied by 100. The germination index was calculated as described in the Association of Official Seed Analysis (1983) by the following formula:

$$\text{Germination index} = \frac{\text{No. of germinating seeds}}{\text{Days of first count}} + \frac{\text{No. of germinating seeds}}{\text{Days of final or last count}}$$

The germination value (GV) was calculated according to Hossain et al. (2005) by the following formula: Germination value = (Σ DGs/N) X GP/10. Where (GP) is the germination percentage at the end of experiment, (DG) is the daily germination speed obtained by dividing the cumulative germination percentage by the number of days since sowing, (Σ DGs) is the total germination obtained by adding every DGs value obtained from the daily counts, (N) is the total number of daily counts starting from the first germination and (10) is constant. Counting of number of leaves was done at the end of experiment (45 days after seed sowing) when the true leaves have emerged. Stem girth was measured 1 cm from the base of the stem using vernier caliper. Plant height was measured from poly bag top soil surface upto the highest leaf tip by straightening all leaves. Leaf area was calculated by the leaves traced on a graph paper. Number of roots, root length was measured by destructive method of uprooting the plants and taking measurement by standard method. Stem and root were weighed to record stem, root fresh weight, root/shoot ratio, and total weight per plant (g) was recorded at time of transplanting. Survival percent (after transplanting in main field) was recorded by following formula:

$$\text{Survival percent} = \frac{\text{Total survival transplanted plants}}{\text{Total transplanted plants}} \times 100$$

The net return was calculated by subtracting cost of each treatment from the gross return and benefit: cost ratio = Gross income/cost of seedling production. All data was subjected to analysis of variance (ANOVA) to determine significant differences and comparison of means at significant level of 5%.

RESULTS AND DISCUSSION

The results showed that growing media and cocopeat had beneficial effect on seed germination and growth of papaya seedling.

Seed germination parameters

Seed germination parameters of papaya (*C. papaya*) as affected by growing media and use of cocopeat are presented in Table 1. The treatment T_9 was found to be best followed by T_8 with regard to germination behaviour as these media have suitable physical properties and good water holding capacity that supports the germination of papaya seeds (Table 1). Coir dust when mixed with organic manure is the best media as coir dust has good physical characteristics (Garcia and Daverede, 1994) and also successfully tested as a growing medium in ornamentals (Van Holm, 1993). Germination started at the 9.45 and 9.30 days after sowing on vermicompost medium with 2 cm cocopeat (T_9) for both year of experimentation, respectively. Germination continued until the 25.72 and 22.03 days from sowing where no further germination was noticed in both year of experimentation, respectively. For both year of experimentation, the maximum speed of emergence (277.6 and 709.0), highest germination percent (95.27 and 90.15%), highest seed vigour (91.97 and 86.69), maximum germination index (7.15 and 7.22), germination value (17.33 and 33.83) and least time required for imbibition (9.45 and 9.30 days) and minimum germination period (3.70 and 2.75 days) were obtained in vermicompost + sand + pond soil (1:1:1) with 2 cm filling with cocopeat of seedling poly bags (T_9) in both years of experimentation, respectively. The sand + pond soil (1:1) without cocopeat showed the least results in most cases. The vermicompost medium with 2 cm cocopeat allowed increased germination parameters from the beginning to the end of experiment as compared to other media

Table 1. Effect of seedling growing media and cocopeat on the germination parameters of papaya (*C. papaya*).

Treatment	Imbibition period		Speed of emergence		Germination (%)		Germination period		Seed vigour		Germination index		Germination value	
	2008-2009	2009-2010	2008-2009	2009-2010	2008-2009	2009-2010	2008-2009	2009-2010	2008-2009	2009-2010	2008-2009	2009-2010	2008-2009	2009-2010
T_1	16.67	14.67	129.51	125.22	61.70	57.67	9.05	7.35	52.80	57.67	2.00	2.73	1.66	2.21
T_2	14.60	12.60	133.12	205.99	69.14	66.50	7.35	6.60	62.72	65.60	2.81	3.46	3.20	3.79
T_3	12.35	11.75	166.74	285.68	79.36	81.30	6.50	5.25	73.78	77.69	3.70	4.97	5.64	8.02
T_4	16.45	14.25	152.02	156.21	69.45	72.13	7.90	6.65	62.08	67.30	2.94	3.53	2.53	4.20
T_5	13.55	13.62	188.06	366.19	79.70	83.40	5.65	4.10	73.18	80.33	4.23	4.78	5.59	9.84
T_6	11.75	11.42	206.08	445.04	84.65	86.65	4.72	3.17	80.22	85.39	5.30	5.60	9.01	15.92
T_7	14.30	13.38	231.47	273.54	80.33	74.90	6.95	5.35	73.92	73.60	3.86	3.51	4.44	5.59
T_8	11.13	12.12	257.91	603.16	90.13	85.00	4.55	3.60	84.90	81.62	5.69	6.29	10.35	18.64
T_9	9.45	9.30	277.65	709.09	95.27	90.15	3.70	2.75	91.97	86.69	7.15	7.22	17.33	33.83
SEM±	0.377	0.618	5.874	12.833	1.830	2.173	0.264	0.265	1.804	1.853	0.224	0.136	0.278	0.278
CD at 5%	1.117	1.829	17.385	37.979	5.416	6.432	0.783	0.786	5.338	5.484	0.665	0.404	0.822	0.834

combination in both years of experimentation. The reason for the best performance of pond soil and vermicompost are high organic matter content which increases the water and nutrient holding capacity of the medium, which improve the water utilization capacity of plant. Joiner and Nell (1982) found similar results in peat + perlite mixture for Aglaonema and Dieffenbachia. Vermicompost is reported to have bioactive principles which are considered to be beneficial for root growth and this has been hypothesized to result in greater root initiation, higher germination, increased biomass, enhanced growth and development (Bachman and Metzger, 2008) and also balanced composition of nutrients (Zaller, 2007). The higher available well decomposed organic matter (vermicompost) may preserve soil humidity, increase nutrient content and improve soil structure which increase water absorption and maintains the cell turgidity, cell elongation and increase respiration at optimum level, leading to favourable seed sprouting. Vermicompost mixed with pond soil affects properties of soil physics, chemistry and biology, since organic matter acts as glue for soil aggregate and source of soil nutrient (Soepardi, 1983). Vermicompost granules may develop soil aggregate and it's granulating. Soil aggregation will improve permeability and airflow in the polybags. Vermicompost and pond soil (due to high organic matter) may decrease fluctuation of soil temperature. Further, seed germination and root growth becomes easier to the particular depth so that plant grows well and may absorb more water and nutrient (Jo, 1990). Organic matter may also improve nutrient availability and improve phosphorus absorption (Karama and Manwan, 1990). All these factors are favourable for seed germination and ultimate by increase seed germination percent, speed of emergence, seed vigour, germination index, germination value and reduce imbibition period. Combined application of vermicompost and cocopeat in the treatment T_9 showed significant effect on germination, seedling growth and plant biomass probably due to the synergistic combination of both factors in improving physical condition of the media and nutritional factors (Sahni et al., 2008).

Seedling growth and development parameters

Data presented in Tables 2 and 3 show growth and development of papaya seedling as significantly affected by growing media and cocopeat. Significant differences were observed among the different treatments with regard to seedling growth characters and maximum number of leaves was observed in T_9 treatment (10.02 and 9.67) which was at par with T_6 treatment (9.20 and 9.00), respectively, in both years (Table 2). Maximum seedling girth (3.16 and 3.48 mm), highest seedling height (23.43 and 22.67 cm), largest leaf area (349.33 and 329.20 cm^2), longest root length (10.20 and 9.67 cm) and highest fresh weight of plants (5.02 and 4.77 g) were recorded in T_9 treatment in both years of experimentation, respectively. Similarly maximum number of roots per plant was also higher in T_9 treatment (17.20 and

Table 2. Effect of seedling growing media and cocopeat on the growth parameters of papaya (*C. papaya*) at 45 days after seed sowing.

Treatment	Number of leaves		Stem girth (mm)		Seedling height (cm)		Leaf area (cm^2)		Number of roots		Root length (cm)		Fresh weight of plants (g)	
	2008-2009	2009-2010	2008-09	2009-2010	2008-2009	2009-2010	2008-2009	2009-10	2008-2009	2009-10	2008-2009	2009-2010	2008-2009	2009-2010
T_1	4.00	3.07	0.96	1.28	7.20	9.33	18.30	15.14	5.00	6.60	3.01	3.90	0.60	0.63
T_2	6.05	5.13	1.28	1.60	8.45	10.33	33.35	30.30	9.45	10.35	3.55	4.21	0.80	0.79
T_3	7.11	8.25	1.92	2.24	10.45	12.33	51.23	50.37	12.03	12.30	6.02	5.45	1.00	1.02
T_4	7.23	6.00	1.28	1.80	9.05	9.17	41.25	59.45	7.10	7.83	5.25	4.90	0.72	0.65
T_5	8.25	8.15	2.24	2.44	12.20	12.17	84.31	99.00	11.13	9.69	7.37	6.47	1.20	0.80
T_6	9.20	9.00	2.87	2.76	17.45	17.17	134.00	134.30	12.17	12.35	7.60	6.94	1.65	1.23
T_7	8.13	6.63	1.89	2.21	16.43	11.67	126.17	149.12	13.80	12.00	8.15	7.19	2.67	2.17
T_8	9.02	7.60	2.53	2.84	20.18	19.67	216.25	249.25	16.83	15.20	9.45	8.58	3.82	3.30
T_9	10.02	9.67	3.16	3.48	23.43	22.67	349.17	329.20	17.20	16.17	10.20	9.67	5.02	4.77
SEm±	0.318	0.386	0.083	0.119	0.422	0.448	3.457	3.457	0.581	0.536	0.218	0.196	0.046	0.051
CD at 5%	0.941	1.142	0.245	0.353	1.251	1.326	10.232	10.232	1.721	1.587	0.647	0.580	0.137	0.152

Table 3. Effect of seedling growing media and cocopeat on the growth parameters, survival percent, net return and B : C ratio of papaya (*C. papaya*).

Treatment	Fresh weight of shoot (g)		Fresh weight of root (g)		Survival per cent		Root /Shoot ratio		Days required for gaining transplanting size of seedling		Net return (Rs./1000 seedlings)		B : C ratio	
	2008-2009	2009-2010	2008-2009	2009-2010	2008-09	2009-2010	2008-2009	2009-2010	2008-2009	2009-2010	2008-2009	2009-2010	2008-2009	2009-2010
T_1	0.39	0.39	0.21	0.23	77.30	78.12	0.55	0.59	47.35	45.30	805.00	755.0	1.17	1.15
T_2	0.53	0.51	0.27	0.27	80.37	82.14	0.50	0.54	45.30	42.35	1005.0	955.0	1.19	1.18
T_3	0.69	0.68	0.31	0.34	84.35	86.00	0.45	0.50	42.50	41.20	1905.0	1855.0	1.35	1.34
T_4	0.54	0.47	0.18	0.18	81.80	82.60	0.33	0.37	43.60	40.60	1093.0	1043.0	1.20	1.19
T_5	0.95	0.61	0.27	0.19	84.88	86.65	0.28	0.31	40.50	39.50	1893.0	1843.0	1.34	1.33
T_6	1.27	0.96	0.35	0.27	86.86	90.61	0.27	0.28	38.58	37.62	2393.0	2343.0	1.41	1.40
T_7	2.12	1.74	0.50	0.43	87.20	84.30	0.24	0.25	40.25	40.30	2193.3	2148.0	1.65	1.64
T_8	3.11	2.77	0.71	0.60	90.25	89.35	0.23	0.22	38.30	38.35	2993.3	2948.0	1.78	1.77
T_9	4.12	3.98	0.89	0.78	92.23	93.15	0.22	0.20	35.33	35.15	3493.3	3448.0	1.85	1.84
SEm±	0.057	0.057	0.016	0.016	1.633	1.570	0.009	0.009	1.006	1.006	60.324	45.057	0.066	0.066
CD at 5%	0.170	1.170	0.048	0.048	4.835	4.649	0.029	0.028	2.979	2.979	178.53	133.34	0.198	0.198

16.17) which was at par with T_8 treatment (16.83 and 15.20). Highest fresh weight of shoot (4.12 and 3.98 g), fresh weight of roots (0.89 and 0.78 g) and least root/shoot ratio (0.22 and 0.20) was also reported in T_9 treatment in both years of experimentation (2008-09 and 2009-10), respectively. Manure (vermicompost) provides adequate nutrients and enhances both the physical properties and the water holding capacity (Soegiman, 1982). Similar result was also reported by Supriyanto et al. (1990) working on orange seedling where media containing manure produced growth and roots better than those containing sawdust and rice hulk. Purbiati et al. (1994) proved that soil + manure (1:1) was the best medium for the growth components of salacca cv. Pondoh and Bali. Merrow (1995) found similar results in sedge peat medium for *Ravenea rivularis*. Combined application of vermicompost and cocopeat in the treatment T_9 showed significant effect on seedling growth parameters and plant biomass probably due to the synergistic combination of both factors in improving the physical conditions of the media and nutritional factors (Sahni et al., 2008). This result is akin to the findings of Campos Mota et al. (2009) and Abirami et al. (2010) who suggested that since coir dust is low in nutrients when mixed with vermicompost, provides a better growth medium for plant establishment. However, the air filled porosity (AFP), easily available water (EAW) and aeration of vermicompost and FYM were not at the recommended level which in turn limit the root growth and lowered the water holding capacity. Therefore, the medium with vermicompost and cocopeat is more suitable than vermicompost alone because of the better physical properties and enhanced nutrient level.

This treatment combination was also helpful in reducing damping off disease in seedling due to proper aeration in root zone of the seedling and produce highest survival percent of seedling (92.23 and 93.15%, respectively) which was at par with T_8 treatment (90.25 and 93.15%, respectively). Because of the better physical properties and enhanced nutrient level in T_9 treatment, growth of seedling are too fast and minimum days required for gaining transplanting size is 35.33 and 35.15 days which is at par with T_6 (37.62 days) in second year (2009-10) of experimentation (Table 3). Vermicompost with cocopeat may improve soil porosity, water content, pore of drainage, soil permeability and water availability, whereas weight of soil may decrease. This may develop soil aggregation, and moreover it improves permeability and air flow in the soil, this type of condition sharply reduce damping off disease in nursery stage and provide support to fast growth of the seedling due to availability of better nutrition with water and air in root zone of the seedling ultimately, the seedling gain transplanting size very soon in this treatment combination than other treatments. It seems that good physical and biological conditions in cocopeat and vermicompost had positive effect on root development, which is helpful in increased survival percent of seedling in main field after transplanting. Beneficial effect of cocopeat on root system was observed on nutmeg seedling by Abirami et al. (2010), *Osteospermum* cuttings by Nowak (2004), salvia, viola by Pickering (1997) and *Impatiens* by Smith (1995).

Application of vermicompost : pond soil : sand (1:1:1) with 2 cm cocopeat media (T_9) for preparation of papaya seedling proved profitable and showed maximum net return (Rs. 3493.30/1000 seedlings and Rs. 3448.00/1000 seedlings) and benefit : cost ratio (1.85 and 1.84) for the first and second year of experimentation, respectively due to higher germination percent and survival percent obtained (Tables 1 and 3). This treatment was significantly superior to the rest of the treatments during both year but benefit : cost ratio was *at par* with T_8 treatment.

Conflict of Interests

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

Conclusion

In conclusion, presented results showed that vermicompost and cocopeat, due to suitable physical, chemical and biological properties could be used successfully in preparation of papaya seedling. On the basis of results obtained from this study, it is concluded that growing media significantly influenced the germination, growth and development parameters of papaya seedling in which medium of vermicompost + pond soil + sand (1:1:1) with 2 cm cocopeat filling of poly bags was the best media since the germination, seedling growth and development parameters were higher than those on the other media. The overall results revealed that media supplemented with cocopeat gave higher parameters of germination, growth and development of papaya seedling as compared to media without cocopeat. Therefore, this result suggested that vermicompost, pond soil and sand with cocopeat should be used as growing media for higher germination percent quickening of papaya seedling growth and earn more profit by sale of seedlings.

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Extraction, characterization and application of natural dyes from selected plants in Uganda for dyeing of cotton fabrics

P. A. G. Wanyama[1]*, B. T. Kiremire[2] and J. E. S. Murumu[2]

[1]Department of Chemistry, Kyambogo University, Kampala, P. O. Box 1, Uganda.
[2]Department of Chemistry, Makerere University, Kampala, P. O Box 7062, Uganda.

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Cotton fabrics were dyed with natural dyes derived from the crude bark extracts of *Albizia coriaria*, *Morinda lucida, Syzygium cordatum* and *Vitellaria paradoxa* dye-yielding plants collected in 2004 from Mukono and Mbale districts of Uganda. Natural dyes are becoming very important in industry for their less toxic tendencies as compared to synthetic dyes. They are friendly to the environment and hence their increasing demand worldwide. Many new enterprises, of different sizes, have began to cultivate, extract and apply natural dyes. Dyeing was carried out using the simultaneous mordanting method with 10% (o.w.f) of four selected mordants. CIELab coordinates, Chroma (C) and Hue (H) values were found to be dependent on type of mordant and nature of the phenolic composition of the crude extracts. The colour fastness values, both to light and washing, were very good and of textile importance. The colour absorption values (K/S) were calculated using the Kubelka–Munk equation from reflectance values and the same varied from plant to plant due to differences in molecular structures and substantivity to the cotton fabrics. The mordanting process resulted into varied values of colour depth, chroma (C) and hues (H) with significant changes in lightness values (L), and mordant dependent. Colour fastness properties to light and washing were good, between 3 and 4, suitable for textile application.

Key words: Extraction, characterization, natural dyes, selected plants, Uganda.

INTRODUCTION

Dye compounds from natural resources especially from plants are increasingly becoming important alternatives to synthetic dyes for use in the textile industry (Deo and Desai, 1999; Gokhale et al., 2004; Samanta and Agarwal, 2009). Unlike synthetic dyes which have been found to be toxic and harmful to the environment, natural dyes are biodegradable, non-toxic and generally have higher compatibility with the environment when compared with their

*Corresponding author. E-mail: aaronwanyama@gmail.com.

Figure 1. *Albizia coriaria* crude bark material.

synthetic counterparts (Maria et al., 2010). They can provide a wide range of beautiful shades with acceptable levels of colour fastness (Ekrami et al., 2011). Hence there is considerable research work being undertaken across the world on the application of natural dyes in the textile industry and the return to the use of natural dyes as important alternatives to synthetic dyes (Acguah and Oduro, 2012). Reports from India alone highlight an abundance of dye-yielding plants from whose different parts extraction of colour components for textile application and commercialization is gaining prominence in that part of the world (Mayunga, 2007).

Plants have had a long history of use on the African continent, inspiring enormous research efforts to prospect for lead natural products for drug and pesticide development, involving both local and foreign research groups (Mayunga, 2007). However, very little efforts in comparison have been reported in Africa in search for natural dye compounds for textile colouration. The African continent is rich in different plant species with potential to produce novel natural products with dye-yielding properties. Unfortunately, many of these plants remain unknown phytochemically, undocumented and lie in the wild unexploited and yet represent an enormous reservoir of new dye molecules awaiting discovery.

In this paper, extraction of dyes from *Albizia coriaria, Morinda lucida, Vitellaria paradoxa and Syzygium cordatum* selected dye-yielding plants in Uganda, dyeing of cotton fabrics with the extracted dyes and spectral characterization of the dyed fabrics are reported.

MATERIALS AND METHODS

Four potential dye-yielding plants namely; *A. coriaria, M. lucida, V. paradoxa and S. cordatum* were collected in 2004 from Mukono and Mbale districts of Uganda. The well ground crude dye powder of each plant was kept in a properly sealed and well labelled plastic container and kept in a cool place in the chemistry laboratory at Kyambogo University. In this way, the crude plant materials can last for a long time without being degraded by the environment. The plants were identified by Dr. Hafishamana David at the Forestry Research Institute, Nakawa in Kampala Industrial Area.

Extract preparation

Crude bark extracts from *A. coriaria, M. lucida, S. cordatum and V. paradoxa* selected plants were prepared by adding 10 g of the dry and pulverized materials to 250 ml of distilled water in a 400 ml pyrex beaker. The mixture was stirred, heated and maintained and boiled for 2 h, allowed to stand for 20 min and then filtered. The coloured filtrate (200 ml) was used for dyeing cotton pieces of fabric in the presence of 10% (o.w.f) selected mordant.

Dyeing process

Dyeing of cotton fabrics (1.40 g each) was carried out using the simultaneous mordanting method at boiling for 1 h with constant stirring using a fixed amount of liquor ratio (1:200). Dyed samples were thoroughly washed with cold and hot water to remove any unfixed dyed material and finally dried in open air.

Colour measurement and characterization

Colour shades developed during the dyeing of the cotton fabrics were characterized from reflectance measurements using the Datacolour SF 600 spectra Flash reflectance spectrophotometer. The colour values of the dyed fabrics with selected mordants were obtained in terms of CIELab coordinates with illuminant D_{65} with a 10 standard observer. The colour yield (K/S) values with selected mordants were calculated from reflectance measurements using the Kubelka-Munk equation. The amount of light reflected at each wavelength by each dyed fabric sample was plotted as a percentage of the amount of light falling on the coloured surface at each wavelength. The curves thus obtained provided a detailed description of the colour properties of each dyed sample. Colour fastness properties of dyed samples with mordant were evaluated using standard procedures. Light fastness was evaluated by exposing dyed fabric samples to direct sunlight alongside standard blue woolen fabrics for 30 days with a maximum temperature of 26°C.

RESULTS

Natural dyes mostly require a mordant to be fixed onto the textile fibre (Jothi, 2008). Common mordants like alum, potassium dichromate, stannous chloride and ferrous sulphate were used in this study. They had affinity for the dye and the cotton fibers resulting to strong complexes with the dye inside the fibre matrix. The shades of colour developed on the cotton fabrics from the crude dye materials are shown in Figures 1 to 4 were yellow and brown indicate the major involvement of flavonoid and tannin colour moieties during the dyeing process. The results

Figure 2. *Morinda lucida* crude bark material.

Figure 3. *Syzygium cordatum* crude bark material.

Figure 4. *Vitellaria paradoxa* crude bark material.

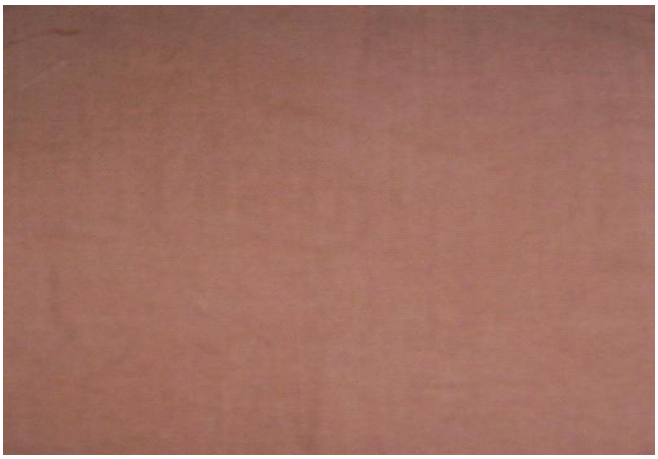

Figure 5. Cotton fabric dyed with *Albizia coriaria* crude bark extract.

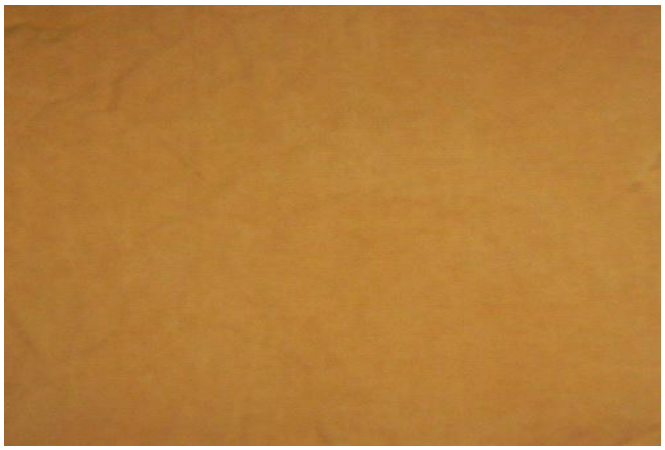

Figure 6. Cotton fabric dyed with *Morinda lucida* crude bark extract.

are shown in Figures 5 to 8. The wash fastness of dye is influenced by the rate of diffusion of dye and state of dye inside the fibre. The dyes extracted from *A. coriaria, V. paradoxa* and *S. cordatum* exhibited good light and wash fastness. The presence of mordant improved the colour fastness and dyeability of the dyes by enhancing interactions between the dyes and cotton fibres. The poor wash fastness for *M. lucida* could be attributed to the presence of smaller dye-metal complexes and weaker dye-fibre interactions as compared to the other natural dyes, thus making them easier to wash out or diffuse from the fibre. This confirms the report that the majority of yellow dyes have poor to light and wash fastness properties (Tim and

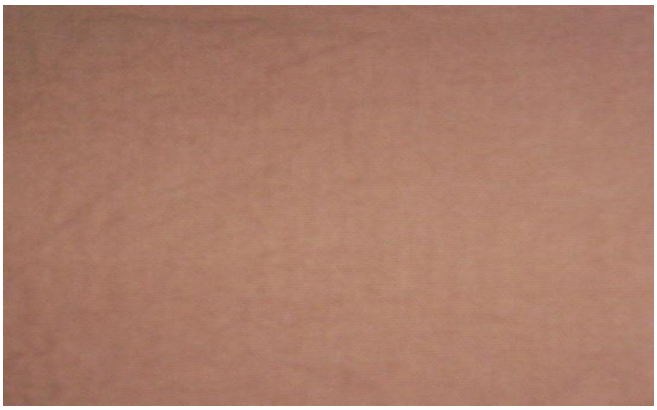

Figure 7. Cotton fabric dyed with *Syzygium cordatum* bark crude extract.

Figure 8. Cotton fabric dyed with *Vitellaria paradoxa* crude bark extract.

Sheila, 1966). The results of colour fastness to light and washing are shown in Table 1. It is possible that the presence of tannin moieties in the crude extracts of *A. coriaria*, *S. cordatum* and *V. paradoxa* enhance their colour fastness by introducing additional hydroxyl and carboxyl functional groups for coordination and stronger dye-fibre-mordant complex formation within the fibre matrix (Wanyama et al., 2011; Ashis and Agarwal, 2009). The effect of selected mordants on colour fastness and dyeability of the cotton material highlights the significant role mordants play in colour characterization of naturally dyed fabric materials. The hue, chroma and light fastness of dyed fabric samples were affected depending on the phenolic composition of the crude plant extracts in this study. The results are shown in Tables 2, 3, 4, 5 and 6. The hue values (H) for each plant sample correlate with the phenolic chemical composition of the crude plant extracts and is mordant dependent.

DISCUSSION

The hues for all the crude plant extracts in this study were from yellowish to red and lying in the first quadrant of the colour space diagram. The amount of light reflecting from each coloured surface was plotted against the amount of light falling on the surface of the dyed fabric samples resulting in characteristic spectral reflectance curves shown in Figures 9 to 13. They represent a permanent record of the reflectance characteristics of the coloured fabric surfaces and vary from plant to plant. The substantivity of the colour components is also represented by the nature of the spectral reflectance curves. Higher substantivity is characterized by low values of reflectance. The values of lightness (L) were very high for all the dyed fabric samples. The values of chroma (C) were low meaning that the dyed fabric materials were all not rich in colour. The colour absorption property of all the dyed fabric samples showed the yellow-red shade range in the visible range of color between 400-480 nm. Potassium aluminium sulphate and stannous chloride mordants exhibited the highest K/S values with *A. coriaria* crude extract (Figure 14); potassium aluminium sulphate and ferrous sulphate exhibited the highest K/S values with *M. lucida* bark and root extracts (Figures 15 and 16), ferrous sulphate, alum and potassium dichromate exhibited the highest K/S values with *S. cordatum* (Figure 17) while stannous chloride and potassium aluminium sulphate (alum) exhibited the highest K/S values with *V. paradoxa* (Figure 18) due to the ability to form strong coordination complexes with the dye molecules. This strong coordination tendency of the mordants in each case enhanced the interaction between the cotton fibres and the dyes, resulting in high dye uptake.

Conclusions

From the results obtained, it could be concluded that natural dyes extracted from *A. coriaria* (bark), *M. lucida* (bark and roots), *S. cordatum* (bark) and *V. paradoxa* (bark) selected dye-yielding plants are of textile importance. The colourfastness properties of the natural dyes investigated indicated potential use in the textile industry. The use of different mordants showed the effectiveness of dye fixation by metal complexation. The specific objectives of the present work were to extract the dyes, characterize them and use them to dye cotton fabrics and the results show

Table 1. Light and washing fastness of selected dye-yielding plants in Uganda.

Local name	Botanical name	Part used	Colour fastness to:	
			Light	Washing
Mugavu	*Albizia coriaria*	Bark	4	3 – 4
Mubajansali	*Morinda lucida*	Bark	3 – 4	2 – 3
Kanziroziro	*Syzygium cordatum*	Bark	3 – 4	4 – 5
Nakunguli	*Vitellaria paradoxa*	bark	3 - 4	4 - 5

Table 2. Effect of selected mordants on colour of cotton sample dyed with *Albizia coriaria* crude bark extract.

Mordant used	Colour coordinates				
	L	a	b	C	H
Potassium aluminium sulphate	69.05	3.35	37.92	38.07	84.96
Ferrous sulphate	57.55	2.08	17.65	17.77	83.27
Stannous chloride	68.42	4.35	31.33	31.63	82.89
Potassium dichromate	67.73	2.84	30.19	30.33	84.62

Table 3. Effect of selected mordants on colour of cotton sample dyed with *Morinda lucida* crude root extract.

Mordant used	Colour coordinates				
	L	a	b	C	H
Potassium aluminium sulphate	58.57	13.26	21.28	25.70	58.08
Ferrous sulphate	21.47	5.92	6.46	8.76	47.13
Stannous chloride	63.98	12.05	14.69	19.00	50.63
Potassium dichromate	68.59	8.22	11.88	14.45	55.31

Table 4. Effect of selected mordants on colour of cotton sample dyed with *Morinda lucida* crude bark extract.

Mordant used	Colour coordinates				
	L	a	b	C	H
Potassium aluminium sulphate	76.24	-0.77	37.52	37.52	91.17
Ferrous sulphate	70.39	3.58	8.59	9.31	67.38
Stannous chloride	80.94	-1.59	14.63	14.72	96.21
Potassium dichromate	75.81	2.68	11.83	12.13	77.24

Table 5. Effect of selected mordants on colour of cotton sample dyed sample with *Syzygium cordatum* crude bark extract.

Mordant used	Colour coordinates				
	L	a	b	C	H
Potassium aluminium sulphate	60.13	8.29	14.72	16.89	60.62
Ferrous sulphate	50.39	5.93	11.34	12.80	62.42
Stannous chloride	61.52	9.40	11.81	15.10	51.50
Potassium dichromate	58.09	8.31	14.13	16.39	59.53

Table 6. Effect of selected mordants on colour of cotton sample dyed with *Vitellaria paradoxa* crude bark extract.

Mordant used	Colour coordinates				
	L	a	b	C	H
Potassium aluminium sulphate	59.43	13.27	19.16	23.31	55.31
Ferrous sulphate	63.75	3.86	7.96	8.85	64.11
Stannous chloride	58.96	9.36	12.21	15.38	52.54
Potassium dichromate	66.05	4.67	14.43	15.16	72.06

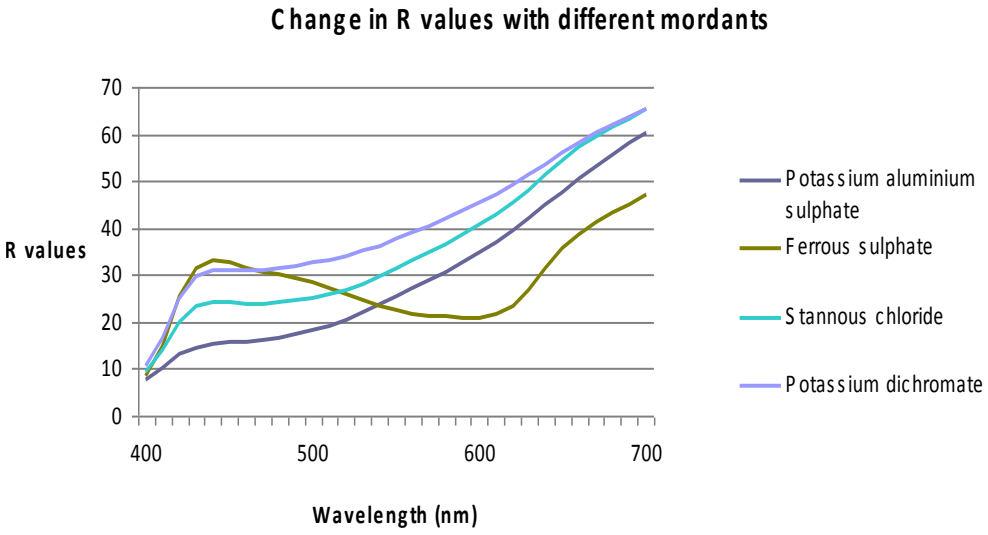

Figure 9. Change in reflectance with selected mordants for cotton fabrics dyed with *Albizia coriaria* crude bark extract.

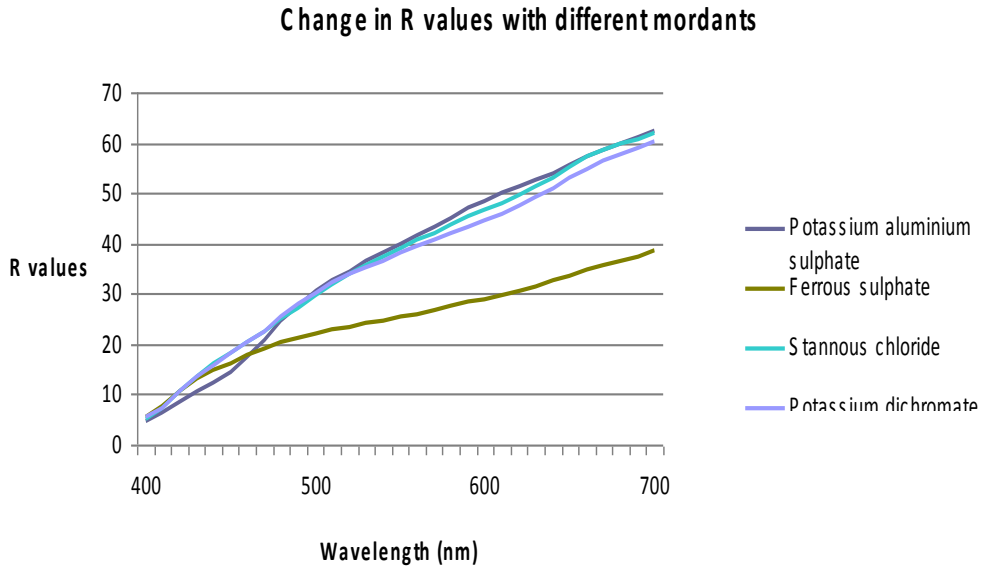

Figure 10. Change in reflectance with selected mordant for cotton fabrics dyed with *Morinda lucida* crude bark extract.

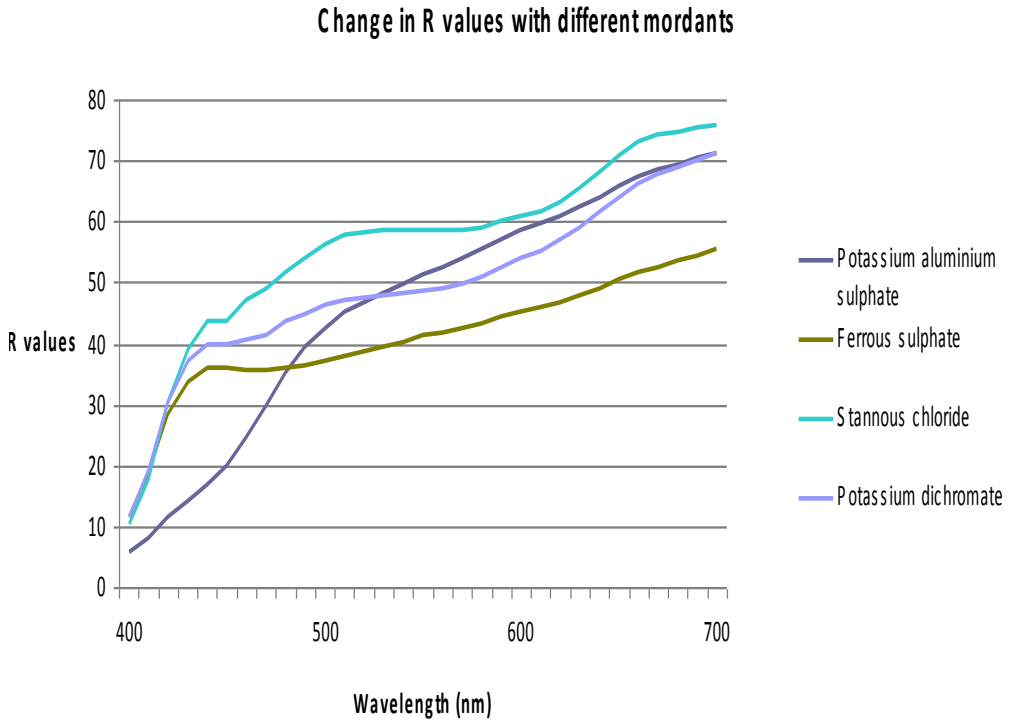

Figure 11. Change in reflectance with selected mordants for cotton fabrics dyed with *Morinda lucida* crude root extract.

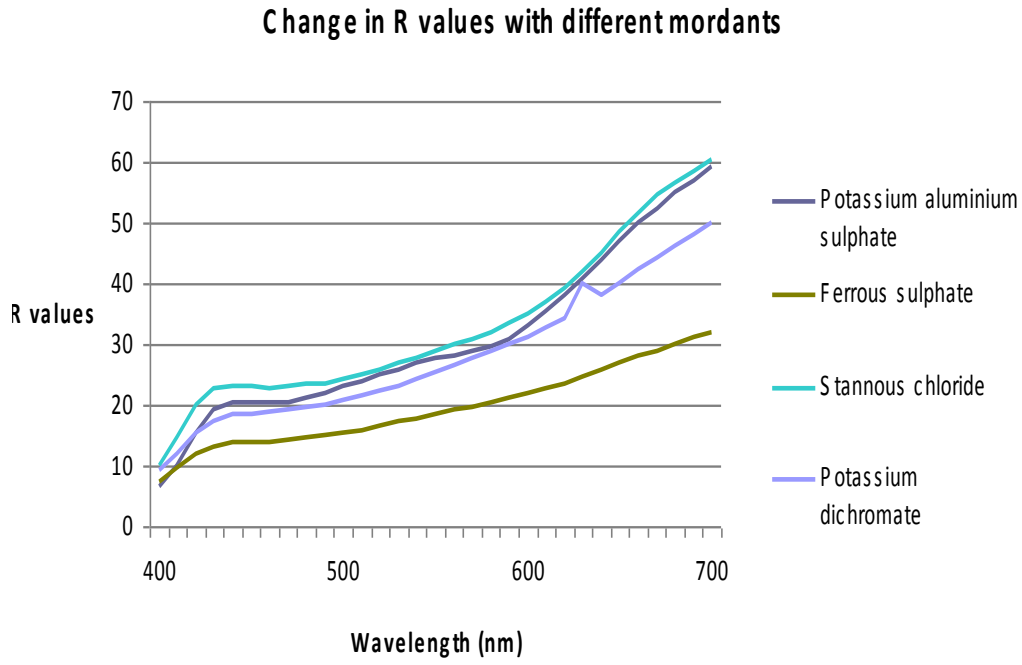

Figure 12. Change in reflectance with selected mordants for cotton fabrics dyed with *Syzygium cordatum* crude bark extract.

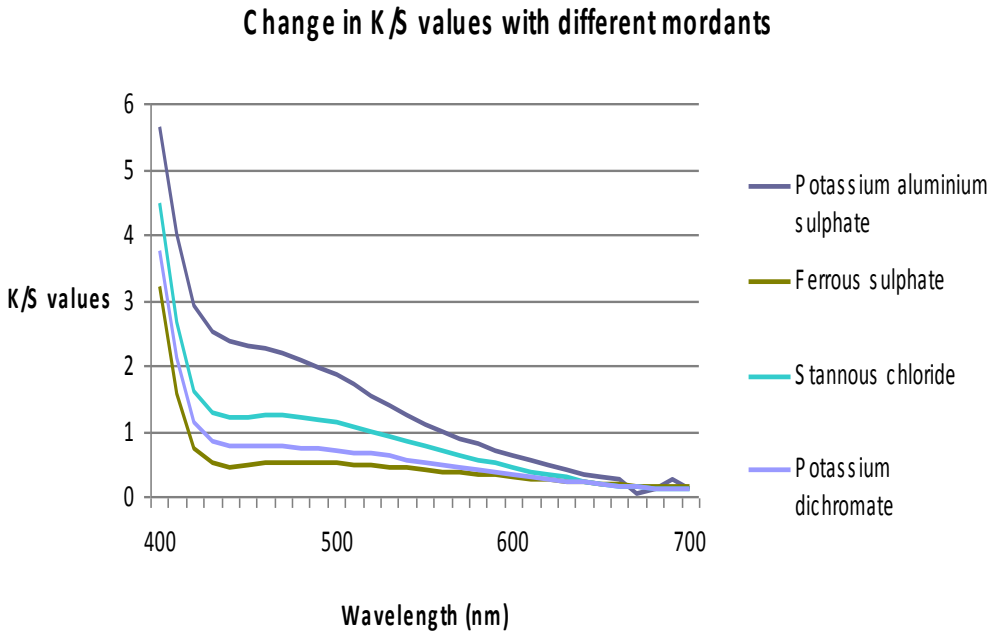

Figure 13. Change in reflectance with different mordants for cotton fabrics dyed with *Vitellaria paradoxa* crude bark extract.

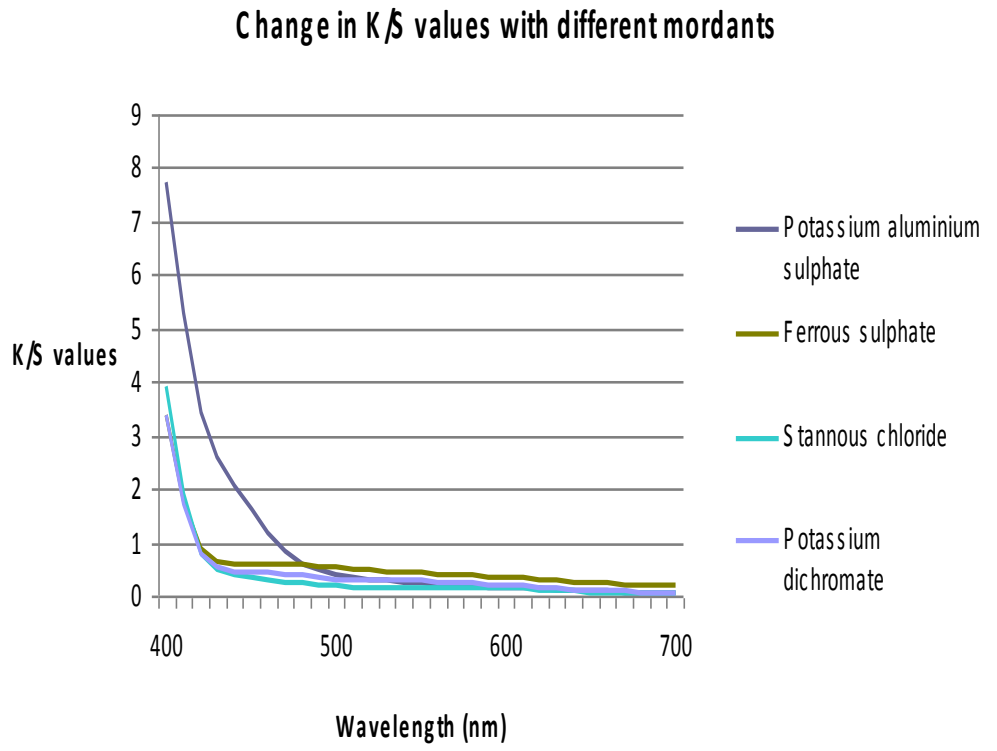

Figure 14. Change in K/S with selected mordants for cotton fabrics dyed with *Albizia coriaria* crude bark extract.

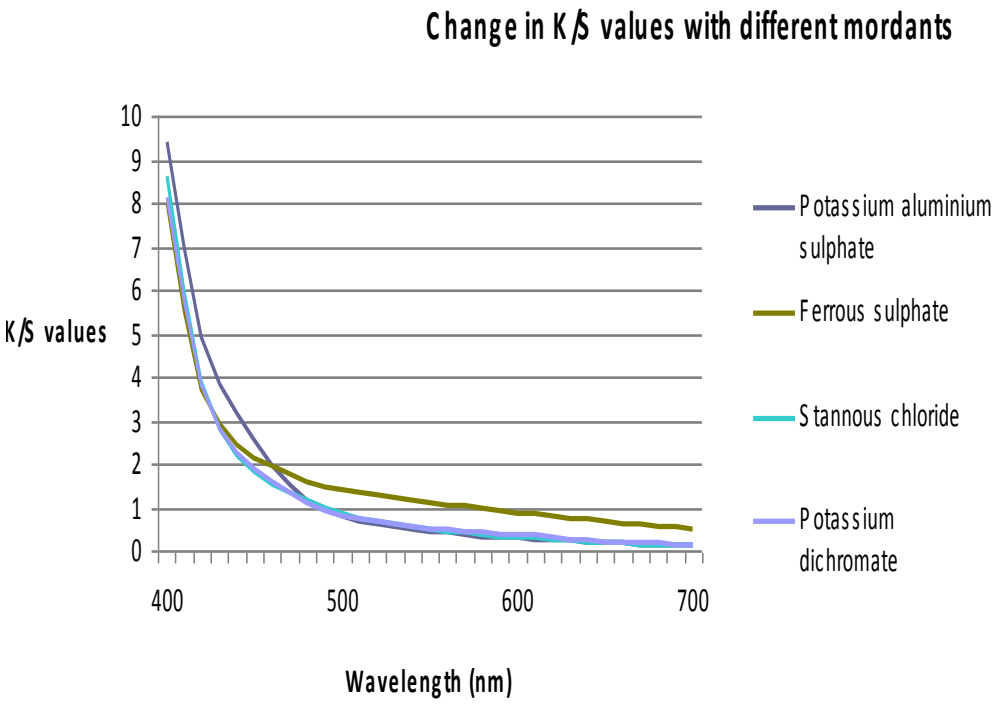

Figure 15. Change in K/S with selected mordants for cotton fabrics dyed with *Morinda lucida* root extract.

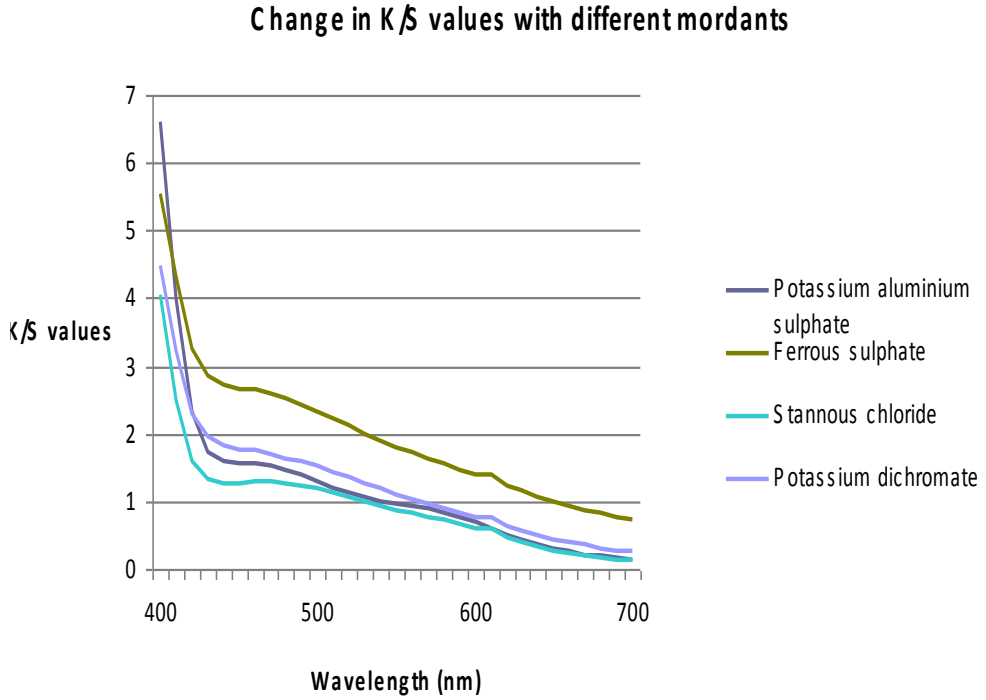

Figure 16. Change in K/S with selected mordants for cotton fabrics dyed with *Morinda lucida* bark extract.

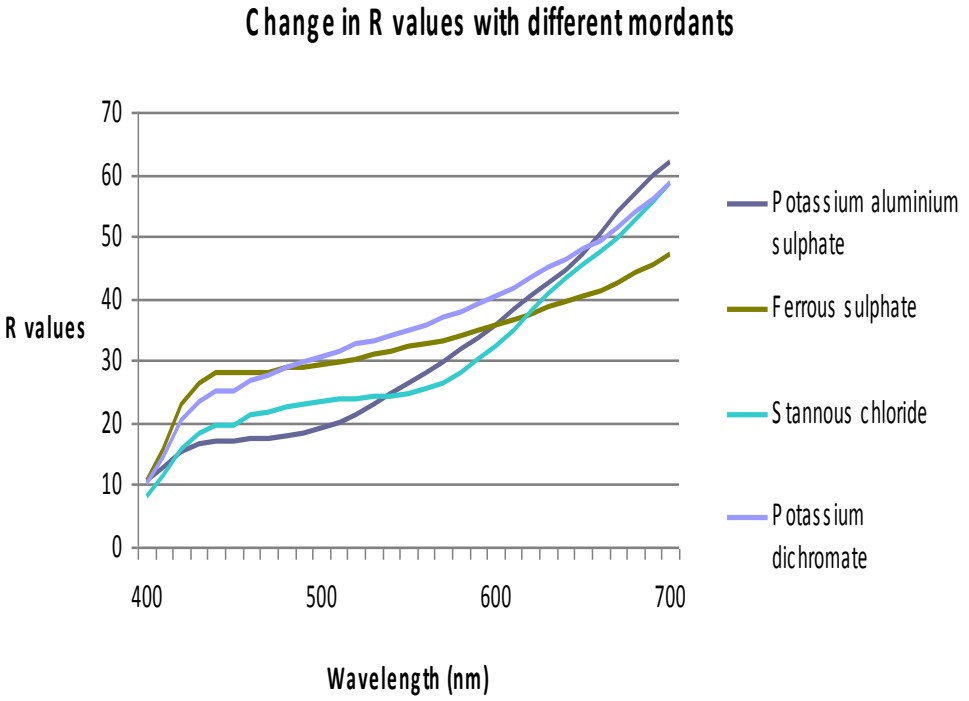

Figure 17. Change in K/S with selected mordants for cotton fabrics dyed with *Syzygium cordatum* crude bark extract.

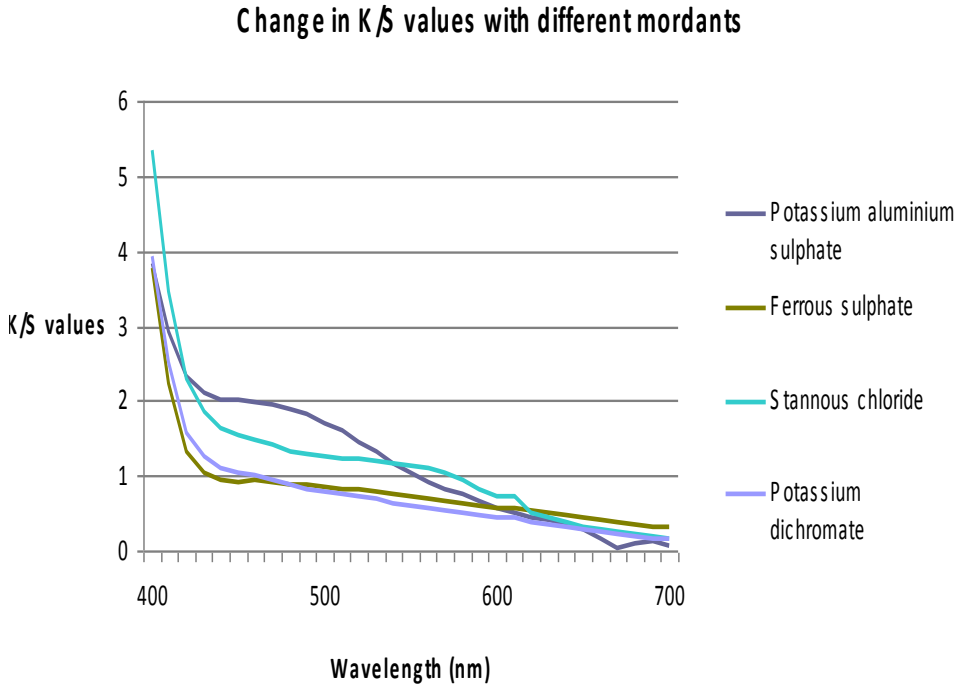

Figure 18. Change in K/S with selected mordants for cotton fabrics with *Vitellaria paradoxa* crude bark extract.

that the objectives were achieved. However, further work needs to be done to determine the chromophoric structures of the major colour components present in all the crude plant extracts that are responsible for the characteristic yellow and brown shades of colour developed on the cotton fabrics

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Full Length Research Paper

Release of cowpea line IT99K-494-6 for yield and resistance to the parasitic weed, *Alectra vogelii* Benth. in Malawi

V. H. Kabambe[1]*, E. D. L. Mazuma[2], J. Bokosi[1] and E. Kazira[3]

[1]Bunda College of Agriculture, P. O. Box 219, Lilongwe, Malawi.
[2]Chitedze Research Station, P. O. Box 158, Lilongwe, Malawi.
[3]Lilongwe Agriculture Development Division, P.O Box 259, Lilongwe, Malawi.

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Field trials were conducted between 2008/09 and 2009/10 seasons to evaluate four new cowpea lines, selected in preliminary studies, for yield, adaptation and resistance to the parasitic weed *Alectra vogelii* in Malawi. The design was randomised complete block design with 4 replicates at research stations and 5 at on-farm sites. For on-farm, villages were the sites and each farmer hosted one replicate. The trial sites were Lisasadzi, Mngwangwa, Bunda College, Chitedze Research Station, Rivirivi and Mpokwa in 2008/09 season and in 2009/10 the sites at Chitedze and Mpokwa were replaced by Mkwinda and Chitala Research Station. Four promising cowpea lines, IT98K-503-1, IT97K-825-15, IT99K-7-21-2-2-1 and IT99K-494-6, all originally from IITA and selected for resistance to parasitic weed *Alectra vogelii* in screen house studies, were compared with released varieties Sudan 1, IT82E-16 and a farmers local entry. The following parameters were evaluated: cowpea grain yield, plants/m^2, seeds/pod, seed weight (g/100 seeds), days to mid-flower, days to maturity, disease scores (scale 1 [clean] – 9 [most severe]) and *A. vogelii* emergence (plants/m^2). The line IT99K-494-6 was found to have comparable or better agronomic traits as compared to control varieties and was eventually released in Malawi in January 2011. The line which is medium maturing has brown grain texture with yield potential of 1-2.0 t/ha. Such a resistant variety is important in *A. vogelii* hot spots to reduce build up in soil seed bank and a viable option for farmers to rotate with cowpeas in order to manage other pests such as *Striga asiatica*, an important parasitic weed, or improve soil fertility through the nitrogen fixation of cowpeas.

Key words: Legumes, witchweeds, parasitic weeds, cowpea, *Alectra vogelii, Vigna unguiculata.*

INTRODUCTION

Cowpea is amongst the important food legumes in Malawi as it is drought tolerant and can do well in warm areas where beans cannot. In 2009/10 growing season, the yields of cowpeas under smallholder farmers in Malawi averaged 378 kg/ha from an area of 61,082 ha (MoAIWD, 2012). Cowpeas are an important source of

*Corresponding author. Email: kabambev@yahoo.com.

Table 1. Test sites, global positioning systems (GPS) and elevation in 2008/09 and '09/10 seasons.

Site	Season	GPS	Masl	On-farm or station	AE+ Zone
Bunda	2008/09; 2009/10	S14°11.096′; E33°46.340′	1194	Station	Mid
Mkwinda	2009/10	S 14°9.066′; E 33°47.312′	1153	Farm	Mid
Lisasadzi	2008/09; 2009/10	S13°15.587′; E33°26.344′	1045	Farm	Mid
Mngwangwa	2008/09; 2009/10	S14°53.284′; E3°42.194′	1133	Farm	Mid
Chitedze	2008/09	S14°0.246′; E33°38.993′	1145	Station	Mid
Mpokwa	2008/09	S15°01.761′; E34° 56.386′	939	Farm	Low
Rivirivi	2008/09; 2009/10	S15°02.704′; E34°54.145′	649	Farm	Low
Chitala	2009/10	S13°41.206′; 34°14.606′	643	Station	Low

AE+ = Agro-Ecological; Masl = meter above sea level.

protein averaging 22 - 34% (Elias et al., 1964; Neil and Brits, 1992; Mahe et al., 1994) and cash for smallholder farmers. The main constraints to its production are insect pests, diseases and lack of suitable varieties (MoAFS, 2005). In 2009/10 growing season, most farmers planted improved cultivar Sudan 1, variety IT82E-16 and local seeds which are preserved and recycled. Potential yields of cowpea is 2,000 kg/ha (MoAFS, 2005). However, *Alectra vogelii*, a parasitic weed for legumes, is also of growing concern particularly as legumes have been widely promoted for some time (ICRISAT/MAI, 2000; Ngwira et al., 2012). The Malawi Government, through the Farm Input Subsidy Program (FISP) also promotes legumes (MoAIFS, 2007). The weed *A. vogelii* is widely seen in Lilongwe and Kasungu plains and many parts of southern region (Riches and Shaxson, 1993; Kabambe et al., 2005; Mainjeni, 1999; Kabambe et al., 2008a, b). In Tanzania yield losses of up to 50% have been reported (Mbwaga et al., 2000). In Kenya, Bagnall-Oakley et al. (1991) reported total crop loss in some parts. In Botswana, Riches (1989) reported losses of 80 - 100% in a susceptible cultivar. Yield losses of up to 15% have been reported in groundnut in Nigeria (Salako, 1984), while in South Africa 30 - 50% reductions in yield of bambara were reported (Beck, 1987). In northern Nigeria, Lagoke (1989) reported that late-sown crops of soybean may be completely destroyed by the parasite. The witchweed *A. vogelii* may be controlled through trap crops such as dolichos bean (*Lablab purpureus* [L.] Sweet) cotton (*Gossypium hirsutum* L.), okra (*Hibiscus esculentus* L.), sunflower (*Helianthus annuus* L.) pea (*Pisum sativum* L.) and faba bean (*Vicia faba* L.) (Parker and Riches, 1993). There is generally greater availability of resistant genotypes against *A. vogelii* (Riches, 2001; Mainjeni, 1999; Rubiales et al., 2006; Omoigui, 2012). Thus, production is threatened by *A. vogelii* and screening for resistance among existing legumes crops or varieties can help to avoid working with the most susceptible varieties. The parasitic weed *Striga asiatica* is also a serious problem in Malawi (Kabambe et al., 2002, 2005), such that the introduction of legume varieties with resistance *to A. vogelii* would be useful to encourage farmers to adopt legumes as trap crops for it or soil fertility replenishment. Also, resistant varieties would slow down the seed build-up of the pest in soils. This study was therefore conducted to evaluate some selected cowpea lines for yield adaptability and resistance to the parasitic weed *A. vogelii*.

METHODOLOGY

Trials design, sites, test materials and management

Four cowpea lines, IT98K-503-1, IT97K-825-15, IT99K-7-21-2-2-1 and IT99K-494-6 were evaluated for yield and resistance to the parasitic weed species *A. vogelii* under field conditions in 2008/09 and 2009/10 in Malawi. These lines were ex-IITA (International Institute of Tropical Agriculture) and were selected for evaluation based on earlier screen house screening for resistance to *A. vogelii* and preliminary yield trials. The lines were compared to released varieties Sudan 1, IT82E-16 and farmers local entry. In both seasons, there were four mid-altitude sites and two low altitude sites. The names, geographic position and altitude of the sites are given in Table 1. The trial design was randomized complete block with four replications at station sites while on-farm there were five replicates, with one farmer hosting one replicate.

Experimental plots had 5 with ridges each 4 m long and 0.75 m apart (gross plot size 15 m^2). No fertilizers were applied to plots. Two seeds of cowpeas were planted at 20 cm apart. Planting was done with first rains in low altitude sites and in mid-January for mid-altitude sites according to standard recommendations (MoAIFS, 2005). There were 4-5 farmers per village or site, making one complete trial.

Data collection and analysis

All crop plant or *A. vogelii* data was collected from the three middle rows (net plot size 12 m^2). Data were collected for evaluation of plant cowpea grain yield (adjusted to 12.0% moisture), plant count/m^2, *A. vogelii* counts/m^2, seed weight, seeds/pod, pod length (cm) days mid-flower and maturity. Counts of *A. vogelii* were taken at time of flowering of cowpeas. Assessment for disease was done at Chitedze Research Station only, where the project pathologist is based. Entries were scored for *Aschochyta* blight (*Ascochyta phaseolorum*), Scab (*Elsinoe phaseoli*) and *Cercospora* leaf spot infection. Disease scores were based on 1-9 scale where 1 was clean and 9 was severe. For infection, the score was the percentage of plants infected. Data were analyzed on site basis

Table 2. Mean monthly rainfall (mm) for the some trial sites in 2008/09 and '09/10 seasons.

Month	Site and season							
	Bunda		Mngwangwa		Rivirivi		Chitedze	Chitala
	2008/09	2009/10	2008/09	2009/10	2008/09	2009/10	2008/09	2009/10
Oct. '09	18.5	0.9	0	0	3.0	16.5	45	0
Nov. '09	119.2	60.5	51	83	113.4	55.4	112	5
Dec. '09	177.1	114.5	147	184	242.1	113.5	116.4	88.1
Jan. '10	291.9	125.1	198	249.5	300.5	222.7	227.8	220.9
Feb. '10	174.0	337.6	152	306	145.1	320.9	121	476.5
Mar. '10	218.3	118.4	134	71	125.1	72.6	223.7	156.2
Apr. '10	28.0	5.4	71	36.5	6.3	34.6	18.1	0
May '10	0	4.7	0	0	0	0	0	0
Total	1026.7	767	753	930	932.5	836.2	864	946.7

Table 3. Effect of genotype on *Alectra* counts m^{-2} taken after flowering at all the trial sites in 2008/09 season.

Entry name	Bunda*	Lisasadzi	Mngwangwa	Mpokwa	Rivirivi	Chitedze
IT98K-503-1	1.35bc	0.50b	3.33	0.6	0.0	0.05
IT97K-825-15	0.08c	0.70b	0.60	0.03	0.08	0.25
IT99K-7-21-2-2-1	0.25c	1.38b	1.46	0.11	0.0	0.17
IT99K-494-6	0.00c	0.60b	1.02	0.04	0.03	0.0
Sudan-1	5.82a	3.56ab	4.03	0.86	0.0	0.58
IT82E-16	6.25a	6.70a	2.86	0.96	0.14	0.0
Farmers' local	2.80b	1.14b	3.29	-	0.03	0.11
Mean	2.36	2.08	2.43	0.44	0.04	0.17
F Prob	<0.001	0.005	0.13	0.28	0.32	0.29
LSD (0.05)	1.59	3.28	3.0	1.0	0.13	0.56

*Means in column denoted by same letter are not significantly different at 5% using LSD.

according to the analysis of variance procedure using the Genstat package. Comparisons between means was done using the least significant difference, LSD, $t_{\alpha} = 0.05$.

RESULTS

Rainfall

In 2008/09 rainfall was normal and fairly distributed for all sites except Mngwangwa and Chitedze, which had relatively poorly rains in February 2009 (Table 2).

Alectra emergence

In 2008/09, the sites at Mngwangwa and Lisasadzi had high infestation of *Alectra*, even though yields were good (Table 3). However, within the plots, spots that had high numbers of *Alectra* caused stunted growth and wilting on cowpea plants. At Mngwangwa, where pressure was high, entries IT99K-7-21-2-2-1, IT97K-825-15 and IT99K-494-6 showed fair resistance. At Lisasadzi, with high *Alectra* pressure the same lines showed good resistance (low support of parasite). At Bunda College, with high *Alectra* pressure, the same three lines showed resistance. On the overall, entries IT99K-494-6 and IT99K-7-21-2-2-1 were best for resistance with maximum Alectra of 1.02 and 1.46 plants m^{-2}, respectively, as compared to maximum emergence of 6.7 and 5.82 plants m^{-2}, respectively for IT82E-16 and Sudan-1, the local checks.

In 2009/2010, entry IT99-494-6 was the most resistant line among the seven entries and was followed by IT97K-825-15 and IT99K-7-21-2-2-1, respectively (Table 4). On the overall, IT99K-494-6 and IT97K-825-15 were the best for resistance with 0.10 and 0.80 plants m^{-2} respectively, and entry IT99K-494-6 have nil *Alectra* at Mkwinda and only 0.04 plants m^{-2} at Lisasadzi. There was no Alectra emergence at Bunda College, Chitala and Riviri.

Grain yields

Tables 5 and 6 show cowpea grain yields from mid-altitude sites in 2008/09 and 2009/10. In 2008/09, the line

Table 4. Effect of genotype on *Alectra* counts m^{-2} taken after flowering at three sites in 2009/10.

Entry	Lisasadzi	Mngwangwa	Mkwinda
IT98K-503-1	2.27	2.5	1.9
IT97K-825-15	0.98	1.1	0.3
IT99K-7-21-2-2-1	0.35	3.5	0.1
IT99K-494-6	0.04	0.2	0
SUDAN-1	2.82	2.8	0.8
IT82E-16	2.04	11.9	0.7
Farmers local	2.80	18.0	1.1
Mean	1.61	5.7	0.7
F prob	0.44	0.06	0.18
LSD (0.05)	3.33	13.3	1.59

*Means in column denoted by same letter are not significantly different at 5% using LSD.

Table 5. Grain yield kg/ha of cowpea entries at mid-altitude trial sites in 2008/09.

Entry	Site			
	Lisasadzi	Mngwangwa	Bunda	Chitedze
IT98K-503-1	2034^{ab}	856	1496	1667^{b}
IT97K-825-15	1527^{b}	796	1276	1083^{b}
IT97K-7-21-2-2-1	1749^{b}	543	1665	1222^{b}
IT99K-494-6	2345^{a}	1114	1846	972^{b}
IT82E-16	1936^{ab}	903	1631	3194^{a}
SUDAN-1	2328^{a}	1070	1728	2278^{ab}
LOCAL	-	536	794	1861^{ab}
MEAN	1936	831	1492	1754
F PROB	<0.025	0.058	0.208	0.03
LSD (0.05)	523	435	830	1340

*Means in column denoted by same letter are not significantly different at 5% LSD.

Table 6. Grain yield (kg/ha) of cowpea entries at mid-altitude trial sites in 2009/10.

Entry	Site			
	Bunda	Lisasadzi	Mngwangwa	Mkwinda
IT98K-503-1	1808^{ab}	1421^{bc}	730^{c}	979^{b}
IT97K-825-15	1102^{d}	658^{f}	361^{e}	434^{d}
IT99K-7-21-2-2-1	1266^{d}	1024^{e}	619^{d}	763^{c}
IT99K-494-6	1528^{cd}	1757^{a}	932^{a}	1195^{ab}
Sudan1	1621^{bc}	1376^{cd}	938^{a}	1027^{ab}
IT82E-16	2076^{a}	1513^{b}	861^{a}	1060^{ab}
Farmers local	1019	1275de	528^{d}	1089^{ab}
Mean	1489	1289	710	935
P	<0.001	<0.001	<0.001	<0.001
LSD 5%	337	111	99	139

*Means in column denoted by same letter are not significantly different at 5% LSD.

IT99K-494-6 gave the highest yields at Lisasadzi, Bunda and Mngwangwa, which were sites with highest *Alectra* pressure. In 2009/2010, the line IT99K-494-6 gave highest or joint highest yields at Lisasadzi, Mngwangwa

Table 7. Grain yield kg/ha of cowpea entries at two low altitude trial sites in 2008/09 and 2009/10 seasons.

Entry name	Site and season		Site and season	
	2008/09		2009/10	
	Rivirivi	Mpokwa	Rivirivi	Chitala
IT98K-503-1	749[b]	461[b]	1165[b]	849
IT97K-825-15	589[b]	447[b]	1064[b]	693
IT97K-7-21-2-2-1	903[b]	353[b]	1328[b]	852
IT99K-494-6	1372[ab]	562[b]	1189[b]	786
IT82E-16	2156[a]	896[ab]	1255[b]	855
Sudan-1	2158[a]	1402[a]	2029[a]	980
Local	964[b]	353[b]	1814[ab]	852
Mean	1270	681	1406[b]	838
LSD	878	544	457	242
F Prob	0.004	0.004	0.002	0.38

*Means in column denoted by same letter are not significantly different at 5% LSD.

Table 8. Effect of genotype on days to 50% flower at 5 sites, 2008/09.

Entry	Mngwangwa	Bunda	Lisasadzi	Mpokwa	Balaka
IT98K-503-1	46[b]	52[b]	57[b]	46[b]	48
IT97K-825-15	47[b]	56[a]	58[b]	53[a]	52
IT97K-7-21-2-2-1	48[ab]	54[ab]	59[b]	53[a]	53
IT99K-494-6	46[b]	52[b]	57[b]	46[b]	47
52=IT82E-16	44[c]	48[c]	55[b]	39[c]	46
53=Sudan-1	46[b]	47[c]	59[b]	41[c]	51
54=Local	50[a]	53[a]	89[a]	-	52
Mean	47	52	62	46	50
P	<0.001	<0.001	<0.001	<0.001	0.80
LSD	2	3	6	3	12

and Mkwinda (Table 6). However, the two released varieties also gave similar high yields. In the low altitude sites Sudan 1 and IT82E-16 gave better yields than IT99K-494-6 (Table 7).

Days to mid-flower and maturity

The number of days to 50% flower in 2008/09 season is shown in Table 8. Plants flowered earliest at low altitude sites as compared to mid-altitude sites. The entry IT82E-16 was the earliest to flower at both mid and low-altitude sites (range 39-55 days, followed by Sudan 1 (range 41-59 days) then entries IT99K-494-6 and IT99K825-15 (range 49-57 days). In general, the maximum difference between flowering dates of the improved varieties was about 10 days. In 2009/10 the trend was similar in terms of variety responses. However, flowering was generally delayed by about 2 days (Table 9). The flowering time can vary depending on planting date which affects temperature exposure during the season. Discussions with farmers revealed that such differences were quite important to them.

The effects of variety on days to maturity are shown in Tables 10 and 11. In 2008/09, plants matured early at Rivirivi and Mpokwa (66 days as compared to the other sites (76-81 days), which was expected as these sites are climatically warmer and drier areas. The entry IT99K-494-6 matured between 71 and 82 days in the mid-altitude sites, as compared to 72-74 days for Sudan-1. In 2009/10 season, the entry IT99K-494-6 matured between 71 and74 days in the mid-altitude sites, as compared to 65 and 68 days for Sudan-1.

Disease incidence and scores

Assessment for disease was done at Chitedze Research Station only in 2008/09. Entries were evaluated for *Aschochyta* blight (*Ascochyta phaseolorum*), scab *(Elsinoe phaseoli*) and *Cercospora* leaf spot infection. The results showed no significant differences amongst

Table 9. Effect of genotype on days to 50% flower in 2009/10.

Entry	Mid altitude sites				Low altitude sites	
	Mngwangwa	Lisa Sadzi	Bunda	Mkwinda	Chitala	Rivirivi
IT98K-503-1	56d	52^{c}	55^{b}	56	50	53
IT97K-825-15	63^{c}	57^{b}	58^{a}	59	51	56
IT97K-7-21-2-2-1	58^{c}	56^{b}	57^{a}	56	49	48
IT99K-494-6	58^{a}	56^{a}	56^{a}	56	49	48
SUDAN 1	54^{d}	52^{c}	52^{c}	53	47	51
IT82E-16	55^{d}	51^{d}	53^{c}	44	48	50
LOCAL	60^{b}	51^{d}	57^{a}	61	48	50
MEAN	58	54	55	55	49	52
P	<0.001	<.001	<.001	0.39	0.10	0.002
LSD (0.05)	2	1	2	15	2.7	5.4

*Means in column denoted by same letter are not significantly different at 5% LSD.

Table 10. Effect of cowpea line on days to maturity in 2008/09 season.

Entry	Mid-altitude sites			low altitude sites	
	Mngwa Ngwa	Bunda	Lisasadzi	Mpokwa	Balaka
IT98K-503-1	79^{b}	82^{b}	73^{b}	64^{b}	61
IT97K-825-15	83^{a}	88^{a}	72^{b}	74^{a}	69
IT97K-7-21-2-2-1	80^{b}	83^{b}	76^{b}	73^{a}	70
IT99K-494-6	80^{b}	82^{b}	71^{b}	65^{b}	60
IT82E-16	74^{c}	75^{c}	60^{c}	59^{c}	66
SUDAN-1	74^{c}	74^{c}	68^{bc}	60^{c}	69
LOCAL	80^{b}	83^{b}	107^{a}	-	68
MEAN	78	81	76	66	66
P	<0.001	<0.001	<0.001	<0.001	0.57
LSD (0.05)	2	2	11	3	13.2

Table 11. Days to maturity of cowpea entries 2009/10.

Entry	Mid altitude sites				Low altitude sites	
	Bunda	Lisasadzi	Mngwangwa	Mkwinda	Rivirivi	Chitala
IT98K-503-1	71^{d}	68^{d}	71^{c}	71^{c}	62^{cd}	72
IT97K-825-15	79^{a}	79^{a}	89^{a}	80^{b}	68^{b}	71
IT97K-7-21-2-2-1	76^{b}	74^{b}	77^{b}	73^{c}	73^{a}	71
IT99K-494-6	74^{c}	71^{c}	73^{bc}	73^{c}	60^{d}	71
IT82E-16	67^{e}	64^{e}	65^{c}	65^{d}	62^{cd}	70
Sudan-1	67^{e}	65^{e}	68^{c}	66^{d}	61^{cd}	70
Local	70^{d}	67^{d}	73^{b}	89^{a}	65^{bc}	71
Mean	72	70	74	74	64	71
P	<0.001	<.001	<0.001	<.001	<.001	0.63
LSD (0.05)	2	3	5	3	5	2.4

entries for early or late evaluation (Table 12). The scores for *Aschochyta* blight showed medium levels at both early and late stages of assessment while scab scores were quite high at the late scoring stage, averaging 6.0, which are close to severe. The infection of *Cercospora* and scores were also high. The high yields of the released varieties reflect a high degree of tolerance and adaptability.

Table 12. Summary of analysis of variance for disease scores at Chitedze Research Station, 2008/09.

Disease and time of scoring	Mean	F Prob	CV (%)
Aschochyta blight early	2.5	0.43	24
Aschochyta blight late	3.4	0.6	21
Scab early	3.7	0.43	44
Scab late	5.9	0.09	19
Cercospora leafspot incidence (%) early	25	0.38	36
Cercospora leafspot incidence (%) late	71.2	0.80	23
Cercospora leafspot infection early	5.1	0.44	22
Cercospora leafspot infection late	6.7	0.14	11

Table 13. Trial means and standard deviation (in brackets) of yield components and other agronomic traits, 2009/10.

Entry	Plants m^{-2}	Pod length (cm)	Seed per pod	Seed weight g/100
IT98K-503-1	10.00 (2.47)	12.96 (1.04)	8.94 (0.90)	13.20 (0.27)
IT997K-825-15	10.07 (2.72)	13.72 (1.26)	10.66 (0.81)	11.69 (0.61)
IT97K-7-21-2-2-1	11.25 (2.35)	14.26 (0.74)	9.96 (0.94)	17.57 (0.34)
IT99K-494-6	10.07 (2.35)	13.08 (0.24)	9.82 (0.35)	12.92 (0.55)
IT82E-16	11.13 (4.38)	17.00 (0.55)	14.48 (0.94)	10.44 (1.00)
Sudan 1	11.47 (1.79)	16.16 (1.15)	13.72 (1.30)	9.84 (0.21)
Farmers local	10.67 (1.44)	16.72 (1.10)	13.94 (1.09)	13.03 (0.56)

Other agronomic traits, yield components and variety description

Trial means for other agronomic traits and yield components for 2009/10 season are shown in Table 13. All entries seemed to have similar plant establishment with a narrow range of 10-11.4 plants per m^2. The released varieties were generally superior on pod length and seeds per pod. The new lines were superior in terms of seed weights per pod.

DISCUSSION

The results are in agreement with other reports which show wide availability in resistant genotypes against *A. vogelii* (Mainjeni, 1999; Kabambe et al., 2008a, Mbwaga et al., 2000; Riches, 1989, 2001; Rubiales et al., 2006; Omoigui, 2012). The results also generally showed that *A. vogelii* is naturally more prevalent in the mid-altitude sites where all entries are better adapted. The nature of these studies did not allow the researchers to determine yield loss due to *A. vogelii* parasitism, however, it is clear that the two local checks IT82E-16 and Sudan 1 were tolerant to it, owing to their high yields although they supported high numbers of *A. vogelii*. In Botswana, Riches (1989) reported losses of 80 – 100% in a susceptible cowpea cultivar. In northern Nigeria, Lagoke (1989) reported that late-sown crops of soybean may be completely destroyed by the parasite. Although three of the new lines showed fair resistance (low support of Alectra), only the line ITK99K-494-6 was most consistent in yield at most sites. While yield is of interest to farmers, continued growing of susceptible cultivars promotes build-up of the seed bank. As *A. vogelii* is a parasite of other common legume crops in Malawi, such as groundnuts and soybean (Kabambe et al., 2008a), it is therefore sound management to reduce build up through planting varieties which support minimal *Alectra*, especially where yield is not compromised as is the case with IT99K-49-6. The *A. vogelii* may be managed through trap crops (false hosts) such as dolichos bean, cotton, okra, sunflower, pea and faba bean (Parker and Riches, 1993; Kabambe et al., 2008b). The same crops are trap crops for managing *S. asiatica* when grown in rotation or intercrops with cereals (Kabambe et al., 2005; Parker and Riches, 1993; Kabambe et al., 2002; Kabambe et al., 2008b; Parkinson et al., 1987; Carsky et al., 1994). By providing *Alectra*- resistant legume varieties to farmers, their choice to incorporate legumes in the farming systems improves. Legumes, including cowpeas furnish nitrogen through biological nitrogen fixation (Bado et al., 2006) and are important for integrated disease management.

The results of days to flower and maturity showed that the two of the new lines (IT99K-494-6 and IT99K825-15)

were slightly late to flower and mature as compared to Sudan-1. There was no clear relationship between maturity period and yield observed. As an example, the variety IT82E-16 and IT99K-494-6 all gave highest yields at Lisasadzi in 2008/09 even though IT99K-494-6 matured at 71 days as compared to 60 days for IT82E-16. In the low altitude sites, however, IT82E-16 gave highest yields in both seasons. Earliness therefore was important in these short season areas. Results on diseases showed that no particular susceptibility or resistance for all entries such that existing pest and disease management practices would be recommended. Thus, the line IT99K-494-6 was recommended and released in January 2011 for the mid-altitude areas of Malawi due to consistence in resistance and high yield.

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Sustainability of maize-wheat cropping system by different legume intercropping and nitrogen level treatments on light distribution, soil temperature and crop productivity

R. K. Naresh[1]*, S. S. Tomar[1], U. P. Shahi[2], S. P. Singh[2], Purushottam[3], B. Singh[4] and Parvinder Kumar[5]

[1]Department of Agronomy, Sardar Vallabhbhai Patel University of Agriculture and Technology, Meerut (U.P), India.
[2]Department of Soil Science, Sardar Vallabhbhai Patel University of Agriculture and Technology, Meerut (U.P), India.
[3]Department of Pathology & Microbiology, Sardar Vallabhbhai Patel University of Agriculture and Technology, Meerut (U.P), India.
[4]Department of Horticulture, Sardar Vallabhbhai Patel University of Agriculture and Technology, Meerut (U.P), India.
[5]Department of Agricultural Meterology C. C. S. H. A. U., Hissar, Haryana, India

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Agriculture designs for cropping system with legume intercropping for increased resource use efficiency, profitability, productivity and reduced adverse environmental impact are urgently required. A three-year field experiment consisting of six cropping treatments applied during both seasons was conducted during 2008-2009 to 2010-2011 to study the effect of nitrogen and legume intercropping with maize for sustainability of maize-wheat cropping systems. Results indicate that the photosynthetic active radiation was at noon reaching nearly 1940 µmol m^{-2} s^{-1} for maize and 1620 µmol m^{-2} s^{-1} for intercropped legumes. Intercropping can increase light interception, shading and reduce evaporation as compared to sole maize. Maize intercropped had higher values of stomatal conductance and leaf temperature than pure crop. The grain yield and yield attributing characters of wheat crop increased significantly under intercropping treatments when compared with wheat only. Wheat yield significantly increased up to 160 kg N ha^{-1}. However, there was no significant increase in yield of maize beyond 120 kg N ha^{-1}. Sole maize-wheat rotation showed a decline in soil organic carbon by 3.7%, while black gram and cowpea intercropping with maize in paired rows (2:2 row ratio) followed by wheat had increased contents of per cent organic carbon in soil as 0.63 and 0.67, respectively, as compared to initial values of 0.54%. Plots treated with intercrops/farm yard manure (FYM) during the rainy season sustained the wheat yield while the control plot showed a decline in wheat yield by 4-9%.

Key words: Sustainability, intercropping, soil temperature, crop productivity.

INTRODUCTION

Traditionally, soil fertility was maintained through the application of farmyard manure. Manure was largely produced from weeds, crop residue and fodder trees that were fed to tethered animals. Currently, farmyard manure

is the primary source of nutrients for maize fields, although the use of fertilizers is growing in importance. Since farmers apply all the manure available on their farms, it is the increased use of fertilizer that is likely to enable increases in maize production in the future. The agricultural scientists has identified chemical fertilizer as major contributing factor to accelerating agricultural growth and has considered it as one of the priority inputs. Fertilizer application per unit area in maize cultivation is lower than in other crops for two reasons. First, most of the maize is produced in the rainy season where transportation of fertilizer is costly. Second, most of the farmers are subsistent, who often do not have cash to buy expensive fertilizer. Multiple cropping (intercropping or mixed cropping) plays an important role in agriculture because of the effective utilization of resources, significantly enhancing crop productivity when compared with monoculture crops (Li et al., 1999). Introduction of a grain legume in cereal-based cropping system aims at increased productivity and profitability to achieve food and nutritional security and sustainability (Swaminathan, 1998). Intercropping is widely accepted as a sustainable practice due to its yield advantage, high utilization efficiency of light and water, pest and disease suppression (Willey, 1979; Zhu et al., 2000). According to Saleem et al. (2011) and Solanki et al. (2011), the intercropping system provides higher cash return to smallholder farmers than growing the monocrops. Most studies on intercropping have focused on resource utilization, including water light (Shackle and Hall, 1984; Donald, 1985) and nutrients (Dhiman et al., 2007), resulting in substantial yield advantage when compared with sole cropping. In the intercrop, the degree of resource complementarily, the total yield and the relative yield between the individual species is determined by both inter- and intra-specific competition, which again is influenced by the availability of environmental resources, the relative frequency of the species and the density of components (Hauggaard et al., 2006). However, the intercropped species might utilize the growth resources more efficiently than sole crops, and resources may thus support a greater number of plants. A number of mechanisms exist by which intercrops utilize resources such as light, water and nutrients more efficiently than the equivalent sole crops (Anil et al., 1998; Ghanbari et al., 2010; Undie et al., 2012).

Human efforts to produce ever-greater amounts of food leave their mark on our environment. Persistent use of conventional farming practices based on extensive tillage, and especially when combined with *in situ* burning of crop residues, have magnified soil erosion losses and the soil resource base has been steadily degraded (Montgomery, 2007). Now, people understand that agriculture should not only be about high yield, but also sustainable (Naresh et al., 2011). Farmers concerned about the environmental sustainability of their crop production systems combined with ever-increasing production costs have begun to adopt and adapt improved management practices which lead to the ultimate vision of sustainable conservation agriculture. Conservation agriculture addresses a concept of the complete agricultural system, combining three basic principles: (1) reduction in tillage, (2) retention of adequate levels of crop residues and cover of soil surface and (3) use of economically viable crop rotations. These conservation agriculture principles are applicable to a wide range of crop production systems. Obviously, specific and compatible management components will need to be identified through adaptive research with active farmers' involvement in contrasting agro-climatic production systems. The objective of this study was to identify the effect of nitrogen addition and different legume intercropping with maize for sustainability of maize-wheat cropping systems.

MATERIALS AND METHODS

An experiment was conducted for maize-wheat system with legume intercropping in farmers participatory mode in the jurisdiction of Sardar Vallabhbhai Patel University of Agriculture and Technology, Meerut (Uttar Pradesh), India, during 2008-09 to 2010-11.Treatments included: Sole maize (60 cm) and wheat control grown with FYM @ 10 t/ha, maize intercropped with blackgram/cowpea in paired rows (30/90 cm) in 2:2 row ratio, normal planted maize (60 cm) intercropped in 1:1 row ratio with pigeon pea/blackgram with pure stand of maize as checked under irrigated condition followed by wheat during *rabi* season with three nitrogen levels (80,120 and 160 kg N/ha), applied to maize as well as to wheat. The climate of the area is semi-arid, with an average annual rainfall of 805 mm (75-80% of which is received during July to September), minimum temperature of 4°C in January, maximum temperature of 41 to 45°C in June, and relative humidity of 67 to 83% throughout the year. In general, the soils of the experimental sites was sandy loam soil texture with medium fertility (86.5 sand, 9.2 silt and 4.3% clay) with pH 7.4 and had 0.54% organic carbon at the time of initiation of the study. Maize and intercrop were sown simultaneously during the 1st fourth night of June in each of the three years. Intercropping treatments were randomly allocated to main plots and N levels to subplots to evaluate treatments in split plot design. Nitrogen as per treatment and a uniform dose of P_2O_5 @ 60 kg ha^{-1} to all the treatments were applied through urea and single super phosphate, respectively. 1/3 nitrogen along with full dose of phosphorus was applied as basal while remaining nitrogen was equally top-dressed at knee high and tesseling stages. During *rabi* season wheat crop was sown during the 2nd fourth night of November in each of the three years.

At maturity, seed and stover/straw samples were analyzed for total N to determine the N uptake. After completion of three sequences, soil samples were analyzed for total N to calculate N-fixation and N balance. Contribution of atmospheric nitrogen

*Corresponding author. E-mail: r.knaresh@yahoo.com.

through legumes was computed by monitoring the changes in total nitrogen as well as that removed by the crop sequences by using the equation:

NF = NR - NA + N/number of leguminous crops

Where, NF = Nitrogen fixed by legume crop (kg/ha), N = change in total N + addition depletion of N in soil (kg/ha), NR = total N removed by sequence (kg/ha), NA = total N applied through fertilizer or manure (kg/ha).

Soil moisture dynamics were measured at 0-20 cm of soil depth during the study in all treatments; using TDR probes (three replications per treatment). The measurement system for the TDR was based on a cable tester (Tektronix 1502C) coupled to a handheld computer (Husky FS/2) (Thomsen, 1994). Soil moisture content during the growing period was calculated for all treatments. The soil layer above thermometers was used to record soil temperatures. The thermometers were buried in the soil horizontally (at 20 cm depth), between two plants in each of the maize and intercrop rows in a middle row. The measurement of temperature in the afternoon was made on relatively clear days.

The fraction of PAR intercepted was calculated by taking ten readings in rapid succession above the canopy and ten readings below the canopy at the soil surface using a Ceptometer. The soil surface measurements were taken by placing the Ceptometer at right angles to the plant rows. Stomatal conductance and leaf transpiration were measured with a portable photosynthesis system (LI-6400 Licor, USA). Measurements were made on the flag leaf for maize and on the central mature leaflet for legume crops.

RESULTS AND DISCUSSION

Photosynthetic active radiation (PAR)

A characteristic pattern of light interception was found for each cropping systems. There was significant difference in light interception between the maize pure stands as compared to the legume intercrop. In light interception by the maize-wheat monoculture increased linearly, reaching about 80% interception for light above ground and for soil moisture is less than in many other intercropping systems. The study showed that maize-wheat intercropping systems had significantly higher energy gain (more radiation intercepted) than pure crop systems. Lower PAR values were observed in Figure 1 in intercropped legumes indicating significant interception by maize leaves during the morning because of planting techniques. The greatest variation between systems occurred at 10 and 16 h when about 960 and 610 µmol m^{-2} s^{-1} were intercepted. The highest values were recorded at noon reaching nearly 1940 µmol m^{-2} s^{-1} for maize and 1620 µmol m^{-2} s^{-1} for intercropped legumes. However, decreases of 1°C in canopy temperature in inter-cropped canopy between 8 and 10 h was observed. The highest canopy temperature and vapour pressure deficit (VPD) were recorded in maize in the afternoon showing high transpiration conditions during the experimentation. Maize stomatal conductance and leaf transpiration in Figure 2 were also positively affected by intercropping. Between 12 and 16 h values recorded were significantly higher for maize intercropping than for sole crop and highest differences were computed at 14 h when stomatal conductance and leaf transpiration of intercropped were 0.218 mol m^{-2} s^{-1} and 7.09 m mol m^{-2} s^{-1}, respectively. In the sole system, these variables reached 0.110 mol m^{-2} s^{-1} and 5.17 m mol m^{-2} s^{-1} , respectively.

Soil moisture content (SM)

Soil moisture content was reduced dramatically in the sole crop of maize due to high evapotranspiration potential; on the contrary, soil moisture content was increased dramatically in the legume intercropping due to low evapotranspiration potential for the growth period (Figure 3). However, comparing maize SM and legume intercropping, the patterns of soil water distribution in the soil profiles differed supporting the null hypothesis. Legume intercropping provided better soil cover as compared to sole maize, so water evaporation at soil surface was low and soil moisture was high as compared to sole maize. Distribution of root systems among species and cropping system influenced the soil moisture down the soil profile. Comparing the soil moisture content of the soil layer, the maize + cowpea intercropping tended to display the lowest differences followed by maize + pigeonpea and the highest difference followed by maize + blackgram, showing intermediate and greater differences (Figure 4). The measured soil moisture contents in the sole maize system were lower than those in the intercropping systems. In intercropping system, water uptake from soil surface layers increased due to increased root density in the upper layers, thus decreasing water dissipated by evaporation. A similar result was reported by Ghanbari et al. (2010).

Growth and yield parameters

Various inter-cropping treatments did vary significantly the growth parameters of maze, namely plant height and dry mater accumulation at 90 days after sowing (DAS) (gm/plant). These parameters also vary significantly with successive increase in nitrogen levels from 80-160 kg N/ha. Mean data for plant height (Table 1) reveals that plant height increased by 8.2 and 14.1% at 80 and 160 kg N/ha respectively, measured under lowest level of nitrogen (80 kg N/ha). During the three years, dry matter produced at 90 DAS was maximum at 160 kg N/ha but remained at par with that accumulated at the preceding level of 120 kg N/ha. Mean data for dry mater accumulation at 90 DAS indicated that dry matter increased by 14.7 and 17.6% at 120 and 160 kg N /ha, respectively over the least dry matter of 102.4 gm/plant obtained under 80 kg N/ha (Table 1).

Intercropping treatments during rainy season had significant variations in spikelets per spike in succeeding wheat crop. Spikelets per spike obtained in the plot

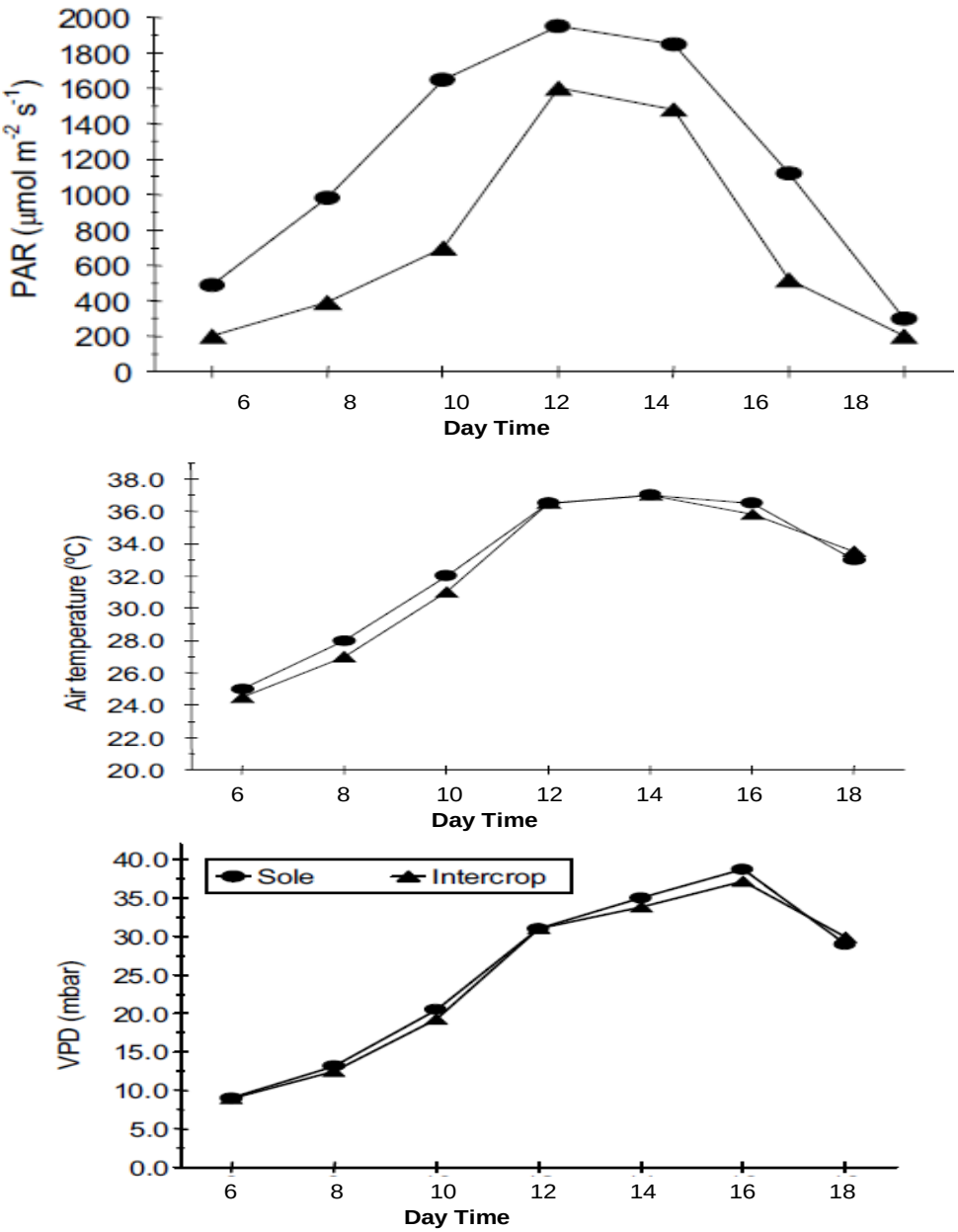

Figure 1. Diurnal fluctuation of photosynthetic active radiations (PAR), air temperature (T) and vapour pressure deficit (VPD) in maize and legumes intercropped.

treated either with FYM or intercrops were significantly superior to that in control plots during respective years (Table 2). Mean data for spikelets per spike calculated for three years indicated that maximum spikelets per spike of 16.4 were counted in the treatment when the crop was raised in the plot where maize + cowpea intercropping was completed, resulting in increase tillers by 3.48% as compared to the control plot (11.8 spikelets/spike). During the three years, increasing levels of nitrogen significantly increased the spikelets per spike till up to 120 kg N/ha. Mean data for spikelets per spike (Table 2) revealed that they increased by 13.8 and 19.4% at 120 and 160 N/ha, respectively, over the least spikelets (12.5 per spike) counted at 80 kg nitrogen level. Shelling percent of maize remained unaffected due to different intercropping treatments in all the three years (Table 2). However, the shelling percent of maize significantly improved with increasing levels of nitrogen during the three years of the study. Mean data for shelling percent (Table 1) revealed that it was improved by 2.20 and 2.60 at 120 and 160 kg N/ha, respectively, over that calculated under 80 kg nitrogen level (71.20). Different intercropping treatments applied in rainy season brought out significant variation in the number of grains per spike and 1000

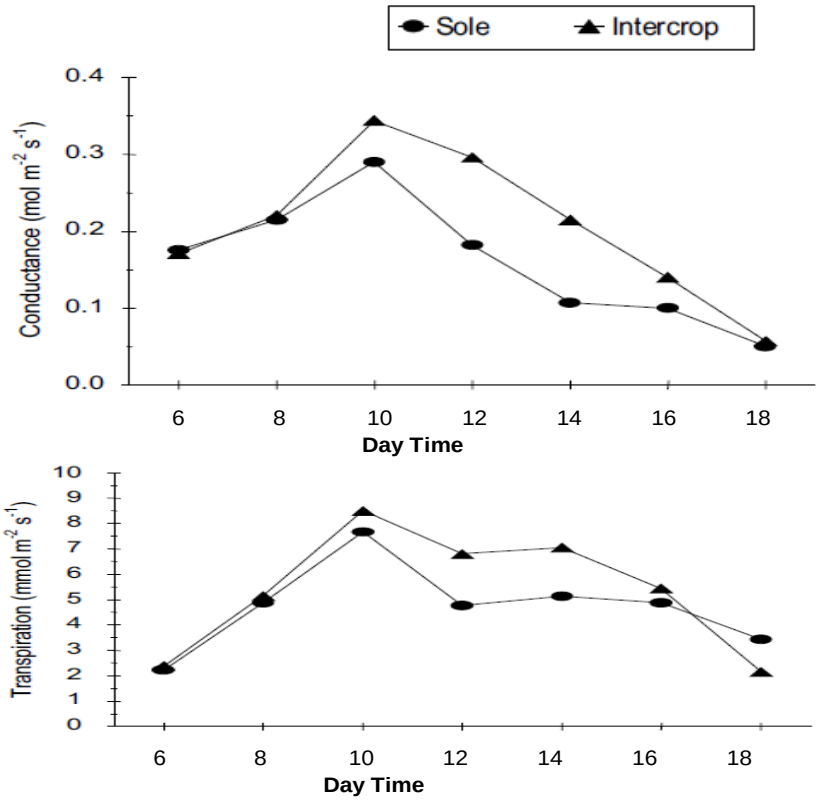

Figure 2. Diurnal fluctuation of stomatal conductance and transpiration of maize sole crop and intercropped with legume.

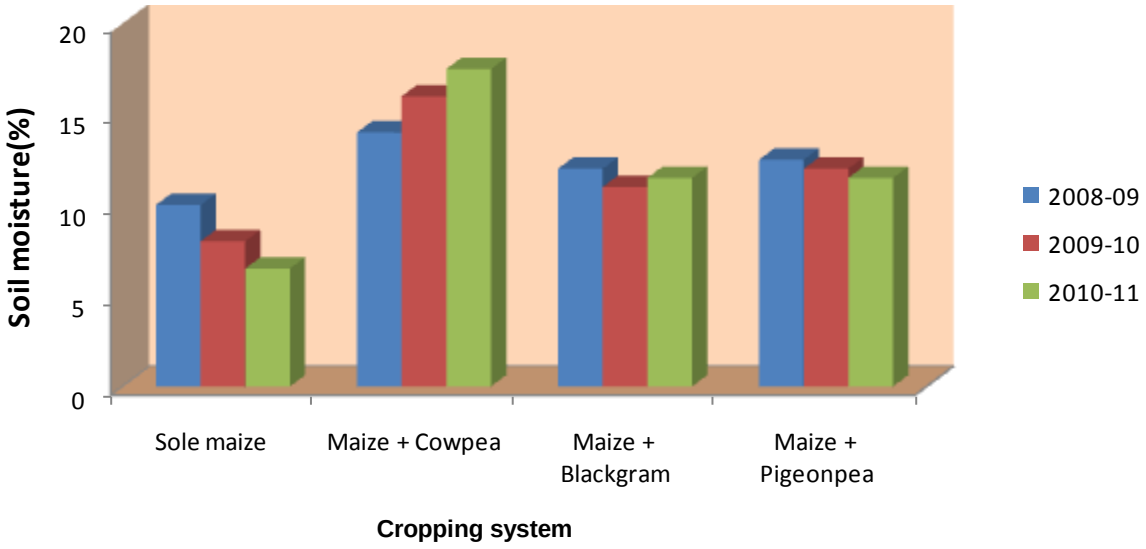

Figure 3. Variation of soil moisture (SM) at different cropping systems.

grain weight in wheat during the years of study.

The number of grains/spike and 1000-grain weight gradually reduced from 56.6 to 42.6 and 42.6 to 35.7 g, respectively, during initial and final years of the study in the control plot where sole maize during rainy season was raised. While in the case of intercropping treatments, numbers of grains/spike and 1000 grain weight under different intercropping treatments were significantly

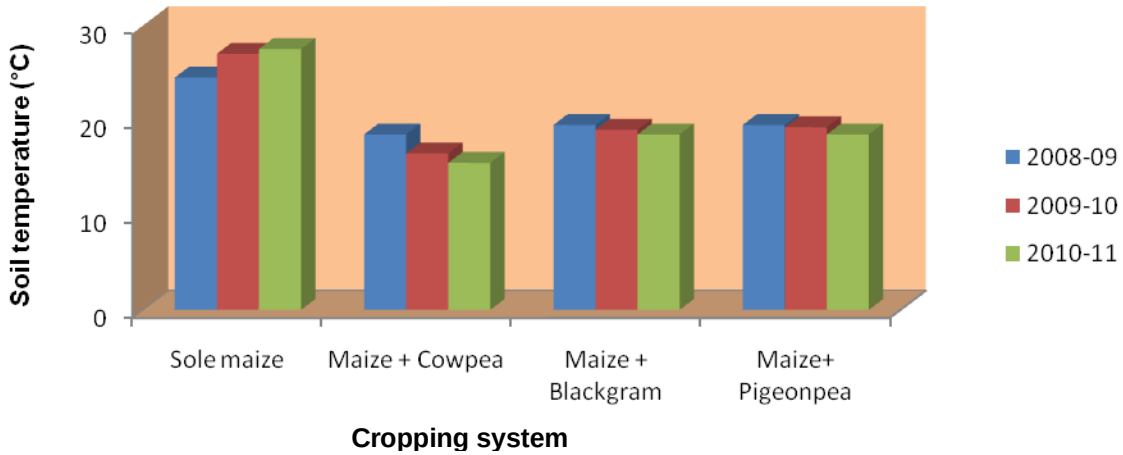

Figure 4. Variation of soil temperature (ST) at different cropping systems.

superior over the control plots but were at par during the third year of the study. Increasing level of nitrogen significantly increased the grains/spike and 1000 grain weight up to 120 kg N/ha. On mean basis, grains/spike increased by 30.8 and 34.0% at 120 and 160 kg N/ha, respectively, over that observed (35.7grains) under initial level of nitrogen. Similarly, 1000 grain weight were increased by 14.2 and 16.4% at 120 and 160 kg N /ha, when compared with those counted (36.2) at initial nitrogen level. These results were in close conformity with that of Solanki et al. (2011) and Undie et al. (2012).

Yield and economic analysis

Maize yield was significantly affected by different intercropping treatments, where pigeonpea intercropping in contrast to pure stand of maize reduced the maize yield significantly. However, the grain yield of subsequent wheat crop was significantly increased due to different intercropping treatments applied to maize. During the first year of the study, wheat yields obtained under the plots treated with FYM (40.35 q/ha) or intercropped with blackgram in 2:2 row ratios (39.64 q/ha) produced significantly superior yield as compared to the control plot (37.82 q/ha). During the following years, the control plot showed a gradual decline in wheat yield by 4.49 and 8.89%, respectively, during 2009-10 and 2010-11. During these years, wheat yields obtained under the plots either treated with FYM or intercrops were significantly higher than that of yield obtained under the respective control plots. This increase in wheat yield might be attributed to nitrogen benefit to soil due to blackgram and cowpea intercropping. Wheat yield significantly increased up to 160 kg N/ha. However, there was no significant increase in yield of maize beyond 120 kg N/ha (Table 3). B : C ratio analysis indicated that the B : C ratio of 2.03 was calculated when maize in paired rows was intercropped with blackgram in 2:2 row ratios (30/90 cm wide beds), followed by wheat. Cowpea intercropping in a similar fashion was the next best treatment in sustaining productivity of wheat as compared to the control. These results are in conformity with the findings of Shah et al. (1991).

Nutrient studies

Organic carbon contents varied with different inter-cropping treatments (Table 3). After completion of the study, the control plot, having pure maize-wheat rotation, showed a decline in soil organic carbon of 3.70%, while blackgram and cowpea inter-cropping with maize in paired rows in 2:2 row ratio followed by wheat increased content of organic carbon in soil as 0.63 and 0.67%, respectively, as compared to initial values of 0.54%. Total nitrogen content of soil also increased with increasing level of nitrogen (Table 4) in all the intercropping treatments. The highest total nitrogen (1399 kg/ha) was found under maize + cowpea (paired rows in 2:2 row ratios (30/90cm)) wheat treatment with net positive N balance of 139 kg/ha less than 160 kg N/ha level. This increase in N availability might be due to the decaying of nodules in legumes. These results are in line with the findings of Saleem et al. (2011) and Naresh et al. (2011).

From the above, it can be concluded that there are opportunities for improving the productivity of maize-wheat system with legumes intercropping in the western Uttar Pradesh, India. Intercropping is the best cropping system because in this system, light interception, soil moisture, soil temperature and yields were higher as compared to sole crops. Microclimatic variations in intercropping system had caused favourable environmental conditions for growth and high yield as compared to sole crops. The legume-maize inter-cropping exploited more soil moisture than a sole maize crop; the beneficial effects of the inter-cropping appeared to greatly compensate for the interception and uptake

Table 1. Growth and yield attributing characters of maize as affected by legume intercropping and N levels.

Intercropping treatments	Growth and yield attributes											
	Plant height (cm) at harvest				Dry matter at 90 DAS (g plant^{-1})				Shelling (%)			
	08-09	09-10	10-11	Mean	08-09	09-10	10-11	Mean	08-09	09-10	10-11	Mean
T_1 Sole maize (60 cm narrow beds)- wheat (control)	260.7	258.3	269.8	262.9	120.7	116.4	123.6	120.2	74.1	73.9	72.3	73.4
T_2 Sole maize (60 cm narrow beds with FYM @ 10t/ha-wheat	277.9	271.2	276.3	275.1	132.7	128.9	136.2	132.6	71.8	72.9	73.6	72.8
T_3 Maize + blackgram paired row in 2:2 row ratio (30/90 cm wide beds)-wheat	253.7	249.6	267.4	256.9	106.2	112.7	119.4	112.8	71.7	73.2	72.8	72.6
T_4 Maize + cowpea paired row in 2:2 row ratio (30/90 cm wide beds) –wheat	251.6	257.9	258.3	255.9	98.7	114.3	121.6	111.5	73.1	72.6	72.9	72.9
T_5 Maize + pigeonpea alternate rows in 1:1 row ratio (30/30 cm flat beds) – wheat	248.7	253.5	256.7	253.0	118.5	123.4	126.8	122.9	72.3	71.8	72.6	72.2
T_6 Maize + blackgram alternate rows in 1:1 row ratio (30/30 cm flat beds) – wheat	242.2	251.8	257.6	250.5	92.3	109.6	117.9	106.6	71.3	72.6	72.3	72.1
C D at 5%	6.8	9.3	8.6	-	3.2	5.8	4.7	-	NS	NS	NS	-
Nitrogen levels (kg/ha)												
80	229.7	240.3	249.2	239.7	95.2	103.5	108.4	102.4	71.2	71.5	70.8	71.2
120	254.1	265.4	267.9	262.5	109.3	123.4	127.7	120.1	72.3	73.4	72.8	72.8
160	267.3	274.1	271.6	271.0	112.6	128.6	131.5	124.2	72.4	73.7	73.2	73.1
C D at 5%	3.4	4.09	2.41	-	3.6	6.11	3.84	-	0.73	0.69	0.58	-

Table 2. Yield attributing characters of wheat as affected by legume intercropping and N level.

Intercropping treatments	Yield attributes											
	Spikelets spike^{-1}				Number of grains spike^{-1}				1000- Grain weight in (g)			
	08-09	09-10	10-11	Mean	08-09	09-10	10-11	Mean	08-09	09-10	10-11	Mean
T_1 Sole maize (60cm narrow beds)- wheat (control)	11.7	11.4	12.3	11.8	42.6	43.9	44.6	43.7	35.7	36.6	37.8	36.7
T_2 Sole maize (60cm narrow beds with FYM @ 10t/ha-wheat	12.8	12.1	13.6	12.8	44.7	45.3	45.8	45.3	36.1	38.3	36.7	37.0
T_3 Maize+blackgram paired row in 2:2 row ratio (30/90 cm wide beds)-wheat	14.3	14.7	15.6	14.9	52.6	53.1	55.3	53.7	41.3	42.7	42.4	42.1
T_4 Maize+ cowpea paired row in 2:2 row ratio (30/90 cm wide beds)-wheat	14.8	15.3	16.4	15.5	53.8	54.1	56.6	54.8	42.7	41.9	42.6	42.4
T_5 Maize + pigeonpea alternate rows in 1:1 row ratio (30/30 cm flat beds) – wheat	13.2	13.8	14.6	13.9	48.7	51.0	53.4	51.0	41.3	41.6	42.4	41.8

Table 2. Contd.

T_6 Maize +blackgram alternate rows in 1:1 row ratio (30/30 cm flat beds) – wheat	11.9	12.3	12.8	12.3	48.5	49.0	52.5	50.0	39.5	40.8	41.3	40.5
C D at 5 %	1.08	1.27	0.93	-	4.06	6.59	4.35	-	0.94	1.29	1.65	-
Nitrogen levels (kg/ha)												
80	11.1	13.3	13.2	12.5	31.3	36.2	39.7	35.7	35.3	36.8	36.4	36.2
120	13.4	14.7	15.5	14.5	50.6	51.5	52.8	51.6	41.3	42.5	42.8	42.2
160	14.8	15.2	16.4	15.5	52.7	53.4	56.3	54.1	42.9	43.2	43.7	43.3
C D at 5 %	1.54	0.65	0.97	-	2.61	2.11	3.84	-	2.06	1.52	1.44	-

Table 3. Grain yield and net monetary returns, and soil organic carbon of maize-wheat cropping system as affected by legume intercropping and N levels.

Intercropping treatments	Grain yield (q/ha)								B:C ratio	O.C. (%)
	Maize and intercrops				Wheat					
	08-09	09-10	10-11	Mean	08-09	09-10	10-11	Mean		
T_1 Sole maize (60cm narrow beds)-wheat (control)	39.13	40.05	40.95	40.04	37.82	36.12	34.46	36.13	1.92	0.52
T_2 Sole maize (60cm narrow beds with FYM @ 10t/ha-wheat	42.60	43.45	45.60	43.88	40.35	40.19	41.40	40.65	1.87	0.58
T_3 Maize+blackgram paired row in 2:2 row ratio (30/90 cm wide beds)-wheat	41.85 (2.85)*	42.75 (3.85)*	43.15 (4.45)*	42.58 (3.72)	39.64	38.00	37.90	38.51	2.03	0.63
T_4 Maize+ cowpea paired row in 2:2 row ratio (30/90 cm wide beds)-wheat	40.42 (3.65)*	41.40 (4.35)*	42.65 (5.25)*	41.49 (4.42)	37.95	39.10	38.35	38.47	2.01	0.67
T_5 Maize + pigeonpea alternate rows in 1:1 row ratio (30/30 cm flat beds) – wheat	34.50 (3.24)*	36.65 (4.45)*	37.60 (5.80)*	36.25 (4.49)	38.45	38.30	37.75	38.17	1.98	0.51
T_6 Maize + blackgram alternate rows in 1:1 row ratio (30/30 cm flat beds) – wheat	38.46 (2.82)*	39.35 (3.75)*	40.85 (5.65)*	39.55 (4.07)	39.40	38.30	37.20	38.30	1.96	0.61
C D at 5 %	2.54	2.08	3.15	-	1.92	2.16	2.35	-	-	0.049
Nitrogen levels (kg/ha)										
80	24.60 (2.60)	27.35 (2.95)	28.20 (3.15)	26.72 (2.90)	28.25	35.20	32.15	31.87	1.83	0.56
120	40.35 (3.85)	41.85 (4.25)	42.45 (4.85)	41.55 (4.32)	42.80	38.95	39.85	40.53	1.99	0.59
160	41.35 (3.60)	42.80 (4.45)	43.75 (4.95)	42.63 (4.33)	45.80	41.15	41.65	42.87	2.08	0.61
C D at 5 %	1.65	2.82	3.73	-	2.14	2.32	2.23	-	-	N.S.
										0.54**

*Figure in parentheses indicate yield of intercrops, **Initial value.

Table 4. Balance sheet of total nitrogen in maize + intercrops-wheat cropping system (kg/ha).

Treatments	Nitrogen levels (Kg/ha)	Mean N removed by crops	Soil N at initiation + N added –N removed	Estimated N after cropping (3 years)	Net soil N balance (Kg/ha)
T_1 Maize (narrow beds) - wheat (control)	80	205	1037	1218	-56
	120	259	1176	1291	+24
	160	298	1255	1337	+56
T_2 Maize (narrow beds)with FYM @ 10t/ha-wheat	80	229	1010	1206	-47
	120	297	1137	1295	+19
	160	367	1249	1367	+89
T_3 Maize+blackgram paired rows in 2:2 row ratio (30/90 cm wide beds)-wheat	80	248	1135	1287	+16
	120	305	1205	1327	+63
	160	348	1321	1388	+121
T_4 Maize+ cowpea paired row in 2:2 row ratio (30/90 cm wide beds)-wheat	80	253	1174	1304	+43
	120	318	1216	1343	+71
	160	353	1345	1399	+139
T_5 Maize + pigeonpea alternate rows in 1:1 row ratio (30/30 cm flat beds) – wheat	80	247	1024	1232	-41
	120	323	1145	1301	+47
	160	364	1299	1384	+119
T_6 Maize +blackgram alternate rows in 1:1 row ratio (30/30 cm flat beds) – wheat	80	251	1046	1240	-23
	120	341	1129	1298	+52
	160	378	1221	1346	+93

losses near the legume crops canopy. Intercropping improves water relations as compared to the sole crops and intercropped maize is more competitive than cowpea in terms of use of resource uptake, mainly soil moisture. In intercropping system, water uptake from soil surface layers increased due to increased root density in the upper layers, thus decreasing water dissipated by evaporation. However, it is evident from the results that legumes and legume-maize intercropping are more effective in improving soil moisture and decrease evaporation from the soil surface due to shading and increase the soil moisture potentially available for transpiration and growth as compared to sole maize.

A yield advantage in intercropping is achieved only when components crops do not compete for the same resources over the same time and space. In these experiments, the degree of below ground competition was not determined, so it is only possible to comment on above ground competition, showing that the sharing of light by the component crops was important for better utilization of resources, resulting in higher productivity of the intercropping system. The maize canopy alone could not utilize all incoming radiation during the growing period, and the remaining solar radiation was captured by the legumes when grown under maize, showing complementarity in use of resources. Maize + blackgram paired row in 2:2 row ratio (30/90 cm wide beds) followed by wheat having a B : C ratio of 2.03 had increased contents of organic carbon in soil as 0.63% as compared to initial values of 0.54%. Nitrogen balance under different intercropping treatments was better. The buildup of soil N (121 kg N/ha) was observed under this intercropping. Thus, blackgram intercropping with maize in paired rows in 2:2 row ratios (30/90 cm

wide beds) followed by wheat was found to be the most sustainable for semi-arid region of western Uttar Pradesh, India.

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Review

Assessment of drought stress tolerance in root and tuber crops

Placide Rukundo[1]*, Hirut Getinet Betaw[2], Stephan Ngailo[3] and Fekadu Balcha[4]

[1]Rwanda Agriculture Board (RAB), P.O. Box 5016, Kigali, Rwanda.
[2]Amhara Agricultural Research Institute, P.O. Box 527 code 100, Bahidar, Ethiopia.
[3]Sugarcane Research Institute, P.O. Box 30031, Kibaha, Coast Region, Tanzania.
[4]Southern Agricultural Research Institute, Hawassa Research Centre, P.O. Box 6, Hawassa, Ethiopia.

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Plant growth and productivity is adversely affected by environmental abiotic and biotic stresses. Drought is one of abiotic stresses that cause serious damage to plants. Therefore, understanding the extent of drought stress and assessing the mechanisms of drought tolerance in plants is very crucial to devise different coping mechanisms with the stresses. Brief discussions have been given on some of the terminologies such as drought tolerance vs. resistance; potential yield vs. yield potential, and water use efficiency. Mechanisms of adaptation to drought stress, inheritance of drought tolerance and the contribution of biotechnology to develop methods of coping up with the problems have been discussed. Finally, methods of screening for drought tolerance have been outlined. The integration of both agricultural practices and conventional and contemporary breeding strategies are necessary in developing crop varieties that are tolerant to abiotic stresses including drought.

Key words: Drought stress, resistance, root and tuber crops, tolerance.

INTRODUCTION

Plant growth and productivity is adversely affected by various abiotic and biotic stresses. Some of the abiotic factors such as low temperature, salt, flooding, drought, heat, oxidative stress and heavy metal toxicity (these stresses) induce various biochemical and physiological responses in plants, which respond and adapt in order to survive and reproduce even under sub-optimal conditions (Barnabás et al., 2008; Mahajan and Tuteja 2005; Seki et al., 2003).

Drought is an extended period of dry weather characterized by a shortage of water supply to plants (Acquaah, 2007). Drought begins when the readily available soil water in the root zone is exhausted (Taiz and Zeiger, 2006). Drought can be permanent, periodic, or random, occurring early, late, or in the middle of the crop season. Drought can also be cumulative or specific and short. Drought in conjunction with coincident high temperature and radiation poses the most important

*Corresponding author. E-mail: rukundoplacide@yahoo.fr. Tel: +250786112423 or +27783930397.

environmental constraints to plant survival and to crop productivity (Chaves et al., 2003; Neumann 2008; Zheng et al., 2009). Further, they are a menace for plants and prevent them from reaching their full genetic potential (Mahajan and Tuteja, 2005). Drought can either be terminal or intermittent (Neumann, 2008). While terminal drought, the availability of soil water decreases progressively and can lead to premature plant death, intermittent drought results from finite periods of inadequate irrigation occurring at one or more intervals during the growing season and is not necessarily lethal. The plant may adjust to short-term water stress by closing stomata and thereby reducing water loss through the leaves. Drought is an extended period of dry weather characterized by a shortage of water supply to plants (Acquaah, 2007). In drought conditions, water potential (Ψw) of soil becomes very negative due to concentration increase of soil solutes. The movement of cell water is determined by the water potential gradient (∆Ψw) that acts as a driving force for transport through a permeable cell membrane (Taiz and Zeiger, 2006). A plant can continue to absorb water only if its Ψw is lower than that of the soil. Indeed, drought stress causes changes in plant cells and tissues to adapt to drought stress condition and continue to acquire little available water from the soil (Bartels and Sunkar, 2005). Symptoms of drought stress start when crop has used between 50 and 80% of available soil moisture (Acquaah, 2007). In fact, the failure of plant to absorb the soil water has severe consequences.

Water plays a crucial role in the life of plant and its availability is a main factor that determines the plant population in the environment (Coley et al., 2009). Water is the main constituent of plant tissues but its quantity varies within plant tissues and plant species. The water content was estimated at 80 to 95% in mass of growing tissues, 85 to 95% in vegetative tissues, 35 to 75% in wood with dead cells, and at 5 to 15% in dried seeds (Taiz and Zeiger, 2006). The distribution of plant species in the environment is associated with their tolerance to environmental stresses (Brenes et al., 2009). Most widespread plant species are drought tolerant (Baltzer et al., 2008; Brenes et al., 2009). A low temperature was suspected to be the main limiting factor of life in the Antarctic environment; however, it was found that the water deficit is the major cause and a positive correlation was observed between the soil moisture and the abundance of plant species (Kennedy, 1993).

Drought is the primary abiotic stress that affects crop production and food availability. In many countries, agriculture depends on rain which in many cases does not meet the crop need (Ober, 2008). The fluctuation of rain affects the growth and production of crop and this causes famines in many semi-arid countries (Acquaah, 2007). Drought can cause the big loss of the production as compared to other isolated biotic or abiotic stress (Boyer, 1982; Ober, 2008). It affects production by reducing the genetic production potential of a plant (Mitra, 2001). Consequently, it is responsible for the difference between the mean yield and the yield potential of a crop and the cause of yield instability in time (Sorrells et al., 2000).

Drought induces physiological, biochemical and molecular changes that have consequences on a plant growth and production (Reddy et al., 2004). The drought osmotic stress causes the removal of water from the cytoplasm to the extracellular space and cell dehydration (Bartels and Sunkar, 2005). Water deficit affects the photosynthetic ability of plants by changing the content and components of chlorophyll, reducing the net CO_2 uptake by leaves, or by decreasing activities of enzymes in the Calvin cycle (Becana et al., 1998; Cornic, 2000; Gong et al., 2005; Lawlor and Tezara, 2009). The osmotic stress of water deficit inhibits strongly the growth of leaves and stem of plants. This has negative effects on the crop yield potential (Westgate and Boyer, 1985). However, the degree of growth inhibition and yield potential reduction depends on the duration and intensity of drought stress, and the genotype of plant species (Bartels and Sunkar, 2005; Monakhova and Chernyad'ev, 2002).

The major cause of reduction of photosynthesis ability and plant growth under drought stress is the disequilibrium between the production of reactive oxygen species (ROS) and their scavenging systems (Becana et al., 1998). Plants under abiotic stresses generate ROS that cause oxidative reactions (Lin et al., 2006). The main ROS are hydrogen peroxide (H_2O_2) and superoxide ($O_2^{\cdot -}$). These ROS are routinely produced in different cellular reactions catalyzed by various enzymes such as lipoxygenase, peroxidase, NADPH oxidase and xanthine oxidase, but the main source of these molecules is the Fenton and Haber-Weiss reactions (Blokhina et al., 2003; Debarry et al., 2005; Lin et al., 2006). The ROS damage lipids, carbohydrates and proteins of cell membrane and cell nucleic acids (Blokhina et al., 2003; Fu and Huang, 2001; Sairam et al., 1997; Zhang and Kirkham, 1996). When a plant is under a serious stress condition, there is an accumulation of ROS because its ROS scavenging and repairing mechanisms of ROS damages are surpassed (Lin et al., 2006). Therefore, a plant must have efficient mechanisms of defense against ROS to survive a severe drought osmotic stress and adapt to drought condition. This paper review concepts of drought stress and methods for assessing drought tolerance in root and tuber crops.

DROUGHT RESISTANCE VERSUS DROUGHT TOLERANCE

Drought resistance refers to the ability of a plant to maintain favorable water balance and turgidity even when exposed to drought condition and its consequences.

When a genotype yields better than another under a severe strain of drought, it is relatively more drought resistant (Blum, 2002). The strain of drought is developed when crop demand for water is not met by the supply, and plant water status is reduced. Plants can resist drought by either dehydration avoidance or dehydration tolerance. Physiological responses of drought resistance depend on magnitude of stress and plant growth stage. For example, drought resistance in seedlings grown in a pot has nothing to do with drought resistance during grain filling in the field (Blum, 2011). Drought tolerance is defined as the relative capacity of plants to maintain functional growth under low leaf water status.

A genotype is drought resistant when it produces an economic crop, within the limits of its production potential under conditions of limited water availability. A genotype can be drought resistant due to the following mechanisms: drought escape, drought tolerance, drought avoidance and drought recovery (Barnabás et al., 2008; Chaves et al., 2003; Ekanayake, 1990). These mechanisms are not mutually exclusive and provide the crop with the ability to resist drought at any given period during its growth cycle (Chaves et al., 2003; Ekanayake, 1990). Plant escape drought by exhibiting high degree of developmental plasticity by being able to complete their life cycle before physiological water deficit occurs; this relies on successful reproduction prior to the onset of severe water stress such as by short life cycle, high rate of growth or the efficient storage and use of reserves for seed production (Barnabás et al., 2008; Chaves et al., 2003). Also, plants endure drought conditions by avoiding dehydration while maintaining tissue water potential as high as possible or tolerating low tissue water potential. This can be achieved either by minimizing water loss or maximizing water intake (Chaves et al., 2003). Water loss can be minimized by closing stomata, reducing light absorbance through leaf rolling, increasing reflectance by dense trichome layer, steep leaf angles or by decreasing canopy leaf area through reduced growth and shedding older leaves (Ehleringer and Cooper, 1992; Larcher, 2000).

Water use efficiency

Water use efficiency is defined by the relationship between grams of water transpired by a crop per gram of dry matter produced (Zangerl and Bazzaz, 1984). Therefore, crops that use water more efficiently produce more dry matter per gram of water transpired, so the efficient use of water is directly correlated to time of stomata opening and stomatal conductance (Zangerl and Bazzaz, 1984). As the plant absorbs CO_2 for photosynthesis, water is lost by transpiration, with variable intensity depending on the potential gradient between leaf surface and the atmosphere, following a stream of water potentials (Shwani et al., 2010).

Yield potential and potential yield

Yield potential is defined as the yield of a cultivar when grown in environments to which it is adapted, with nutrients and water non-limiting and with pests, diseases, weeds, lodging and other stresses effectively controlled (Acquaah, 2007). As such, it is distinguished from potential yield, which is defined as the maximum yield which could be reached by crop in given environments, as determined, for example, by simulation models with plausible physiological and agronomic assumptions (Evans and Fischer, 1999).

Mechanisms of adaptation to drought stress

The interaction and adaptation of plants to environmental signals and stresses is a complex network model (Shwani et al., 2010). Figure 1 highlights the probable physiological, biochemical and molecular responses to drought-stress in higher plants. Plants can withhold the drought stress by dehydration tolerance, dehydration avoidance or drought escape (Ludlow, 1989). In the strategy of dehydration tolerance, it was reported resurrection plants that can survive an extreme internal water deficit. These plants can be still alive when there is 95% of leaf water loss (Scott, 2000). The dehydration avoidance consists of maximizing the water absorption and minimizing the water loss under water deficit conditions. This model is mainly observed on succulent and C4 plant species. The strategy of drought escape is based on a short life cycle. Plants grow and reproduce before appearance of a drought season (Mckay et al., 2003; Mitra, 2001; Passioura, 1996; Richards, 1996). Even though there are these strategies, plants present various changes to resist drought stress. Perennial and deciduous plants reduce their foliage in drought seasons. Plants that are always green present sometimes thick leaves with solid cuticle, deep roots, highly sclerophyllous and reduced size leaves, and effective water use and control of evapotranspiration (Ain-Lhout et al., 2001; Lebreton et al., 1995; Sanguineti et al., 1999; Sorrells et al., 2000; Taiz and Zeiger, 2006). These strategies are usually assisted by others such as accumulation of compatible solutes and increased expression and production of antioxidants. Accumulation of compatible solutes is one of biochemical processes that help plants to survive under drought condition (McCue and Hanson, 1990). Compatible solutes play an adaptive role by osmotic adjustment and protection of cellular compounds (Ain-Lhout et al., 2001; Hare et al., 1998). The compatible solutes are mainly nitrogen containing molecules such as amino acids and polyamines, and hydroxyl compounds. Types of these compatible solutes and the level of their accumulation vary with plant species (McCue and Hanson, 1990). These molecules work together with antioxidants which intervene to eliminate ROS and repair

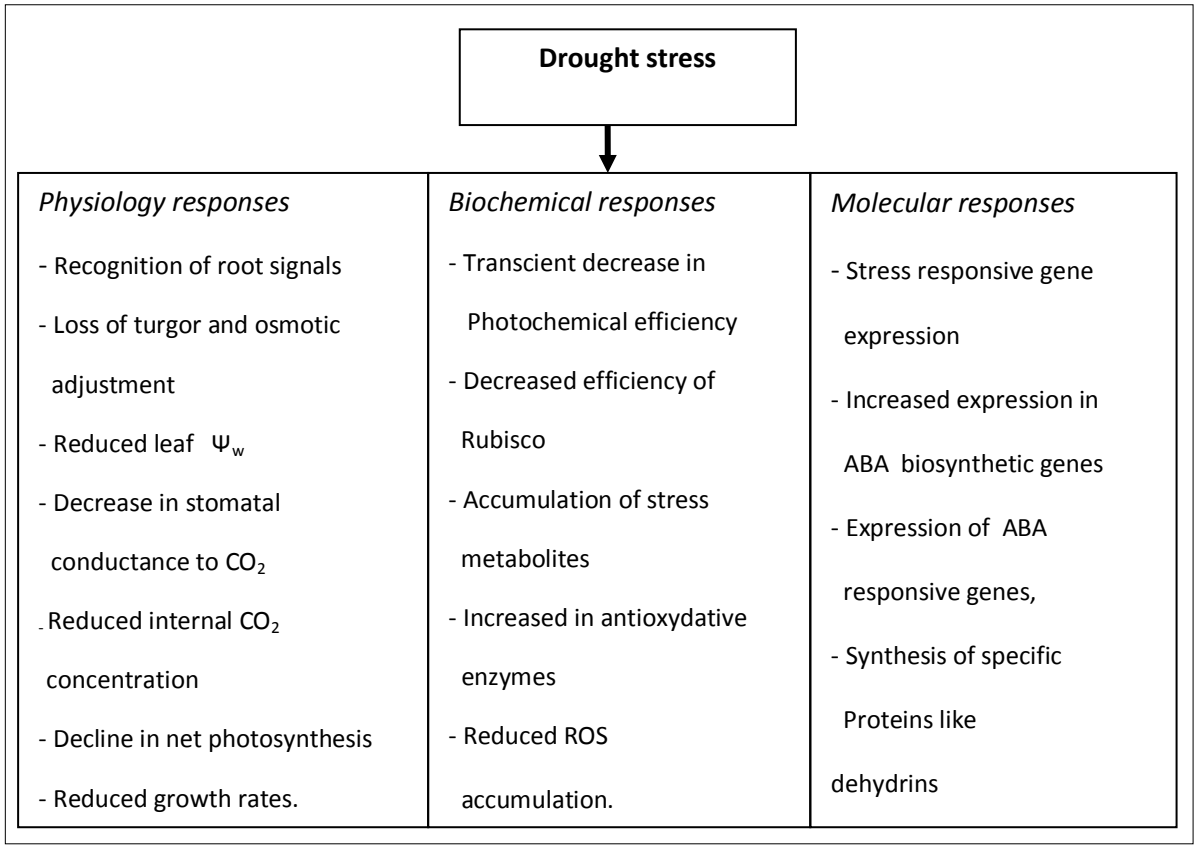

Figure 1. Physiological, biochemical and molecular responses to drought stress in plants (Reddy et al., 2004).

damages of ROS.

Plants produce different antioxidants that have abilities to scavenge ROS. Antioxidants are molecules with small molecular mass such as ascorbic acid, glutathione, tocopherols, phenolic compounds, ROS-interacting enzymes such as superoxide dismutase (SOD), ascorbate peroxidise (APX) and catalase (CAT) (Blokhina et al., 2003; Brosché et al., 2010). These molecules play an important role in controlling the equilibrium between the production and the elimination of free radicals. Moreover, they work in cohesive network reactions and use mainly redox reactions (Lin et al., 2006). Plant varieties that are drought resistant or tolerant express a higher quantity of antioxidants than sensitive varieties (Herbinger et al., 2002; Lin et al., 2006). The quality and the quantity of these molecules are plant species dependent and their expression is affected by plant environmental conditions (Blokhina et al., 2003; Herbinger et al., 2002; Lin et al., 2006). Therefore, understanding the expression mechanisms of these molecules and their function models can assist to identify and develop of drought tolerant varieties.

Adaptation mechanisms to drought stress incriminate plants. Varieties of short life cycle can escape a drought period; but produce low yield (Acquaah, 2007). Mechanisms of dehydration avoidance such as stomatal closure and a decrease of leaf area reduce the assimilation of light and atmospheric CO_2 necessary for photosynthesis (Cornic, 2000; Lawlor and Tezara, 2009). Dehydration tolerance with the accumulation of compatible solutes, the synthesis of antioxidants and the process of ROS scavenging requires too much plant energy. Consequently, these mechanisms reduce the ability of plants to synthesize organic products for stock organs (Mitra, 2001). Thus, the development of a drought tolerant variety such as sweet potato needs to balance all drought tolerance mechanisms without sacrificing the crop productivity (Mckay et al., 2003; Mitra 2001; Passioura, 1996; Richards, 1996).

Inheritance of drought tolerance

Drought tolerance is a complex trait because of the heterogeneity of drought stress in the time and space, and unexpected characteristics of drought stress (Sorrells et al., 2000). The drought tolerance involves actions and interactions of various biochemical, morphological and physiological mechanisms that are controlled by products expressed by different genes (Acquaah, 2007; Mitra, 2001). Moreover, it is difficult to study isolated single gene and to understand its role of drought tolerance in

plant (Mitra, 2001).

Contradiction cases of quantitative nature of drought tolerance were reported in various studies. Ekanayake et al. (1985) have observed that root characteristics are controlled by a qualitative inheritance under a drought condition. The leaf rolling, the osmotic adjustment and the number of roots were identified to be qualitative traits (Mitra, 2001). Study on water deficit mediator genes has indicated that plant species vary in symptoms and reactions to water deficit (Sorrells et al., 2000). The genes responsible for earliness of stem reserves, leaf persistence and dwarfing were identified to be associated with drought tolerance (Foulkes et al., 2007). A drought resistance gene linked with genes for plant height and pigmentation that has pleiotropic effects on a root system was identified in rice and crowpea (Agbicodo et al., 2009; Mitra, 2001; Morgan, 1995). Other proposed candidate genes that are involved in drought tolerance are genes coding for dehydrin proteins that protect cellular components under dehydration condition (Shinozaki and Yamaguchi-Shinozaki, 2007), proteins controlling the equilibrium and damages of ROS (Foyer and Noctor, 2005), proteins involving in osmotic adjustment and plant morphology (Moinuddin et al., 2005; Ober, 2008) and enzymes involved in the accumulation of compatible solutes (Mitra 2001). Indeed, drought tolerance involves many genes which code for products working in a highly coordinated network.

DROUGHT AND BIOTECHNOLOGY

Good genetic maps based on molecular marker technologies are now available for major crops. Many of the traits determining abiotic stress tolerance and the quality and quantity of yield are controlled by a large number of genes, which have only minor individual effects but which act together (quantitative trait loci, QTL) (Barnabás et al., 2008). In crop species with large, complex genomes such as sweet potato, QTL analysis is an important tool in the identification of genetic markers to assist breeding efforts. Barnabas et al. (2008) suggested that correlating genetic information with physiological and morphological traits related to drought tolerance and other traits such as yield will allow the development of new varieties with improved yield safety under water-limited conditions using molecular marker-assisted breeding. The same author highlighted the use of proteomics in investigating the effect of drought and/or heat stress on protein composition as an important step towards understanding the link between environmental factors and plant development. On the other hand, the introduction of isolated individual genes into the germplasm through genetic engineering offers a variety of opportunities to increase environmental stress tolerance in crops (Barnabás et al., 2008). Wahid et al. (2007) applauded the significant contribution of biotechnology in a better understanding of the genetic basis of heat tolerance.

Recent advances in plant genomics have led to the identification of a vast number of potentially beneficial water-stress-related genes, plus technologies for gene overexpression or silencing. Moreover, these can be introduced into transgenic plants under the control of appropriate promoters and are transmitted to subsequent generations (Neumann, 2008).

Screening for drought tolerance

Environmental conditions of study

Study of drought tolerance in plants can be carried out under field or controlled environmental conditions (Acquaah, 2007). The field condition consists of conducting trials under natural conditions. These trials are carried out in the real environments of a plant but it has some limitations of fluctuation of water availability caused by unexpected rainfall. Moreover, environmental factors such as temperature, air humidity and light are variable. Therefore, the screening for drought tolerance is complicated by difficulties of field management (Lafitte et al., 2004). The rainout shelter and *in vitro* techniques were proposed to overcome the limitations of selection for drought tolerance under field condition (Acquaah, 2007). The rainout shelter is a mobile infrastructure that protects plants under experiment from rain. This method controls the uniformity of water supply to plants (Blum, 2002). The *in vitro* approach consists of growing cells or tissues of plant or plantlets on a defined drought stressing culture media under an aseptic and controlled environment (Ahloowalia et al., 2004; Wang et al., 1999). The *in vitro* technique provides precise results but the working environment differs from the natural environment of plant. Therefore, the combination of *in vitro* screening with selection under the natural condition or under the rainout shelter could improve the quality of results.

Screening methods for drought tolerance

Drought stress tolerance is a complex quantitatively inherited and controlled trait. These stresses affect the plant in different ways and induce different genetic responses. Therefore, very careful strategies and powerful methods are required to assess the level of genetic resistance and/or tolerance.

Drought tolerance can be identified by quantifying phenological, morphological, physiological and biochemical characteristics and using molecular tools (Blum, 2002). Phenological and morphological characteristics are mostly used in breeding for drought tolerance. In these approaches data collection consists of measurement of plant growth (size of roots, stem and leaf

Table 1. A scale of visual symptoms of leaf wilting.

Score	Estimation (in %) of turgid leaf area	Description of the symptoms
9	> 95	All leaves turgid
8	80	Lower leaves wilted
7	70	
6	60	Lower half of the plant wilted
5	50	
4	40	
3	30	Top leaves still turgid
1	<5	Plant completely wilted; leaves show necrosis
0	0	

area, gain of fresh and dry weights and yield loss), growth stage (days to flowering and maturity), senescence and leaf rolling (Cheema and Sadaqat, 2004; Spitters and Schaapendonk, 1990). The water content and water potential of plant are indicators used to identify drought tolerant varieties. A variety that maintains its internal water status under a drought stress is considered as drought tolerant. Drought tolerance is also determined by quantifying plant biochemical products such as compatible solutes, chlorophyll, antioxidants and other proteins produced by plant as responses to drought stress (Kasukabe et al., 2006; Reddy et al., 2004; Wang et al., 1999). Diffusion porometry for leaf water conductance, root penetration, distribution and density in the field and infrared aerial photography for dehydration and leaf temperature are used commonly in studies for drought tolerance (Mitra, 2001). Practical methods to measure the drought tolerance are mainly based on phenological, morphological and physiological traits and on biotechnological approaches.

Phenological traits

Growth rate and yield are main phonological traits for determining drought tolerance in crops. The yield reduction due to drought stress, the number of surviving plants and/or a count of plants with tubers are often practiced to study drought tolerance in root crops (Ekanayake, 1990). Data of growth and yield reduction due to drought stress are collected from trial carried out under control and drought stress conditions. These data are transformed into drought tolerance indices such as drought resistance indix (DRI) = (Ys/Yn)/(Ms/Mn), mean productivity (MP) = (Ys+Yn)/2, stress tolerance index (STI) = (Ys)(Yn)/(Mp), stress susceptible index (SSI) = (1-(Ys/Yn))/(1- (Ms/Mp)) and tolerance (TOL) = Yn-Ys. Where Yn: yield of a given genotype in non-stressed environment, Ys: yield of a given genotype in drought stressed condition, Ms: mean yield over all genotypes in stressed environment, Mn: mean yield over all genotype in non-stressed environment (Golabadi et al., 2006; Talebi et al., 2009). These drought tolerances indices assist to identify drought tolerant genotypes within germplasm collections

Morphological traits

Leaf wilting

Visual symptoms of leaf wilting (Table 1) are recorded on a scale base and the measurement unit is expressed in cm s^{-1}. But this might be very subjective since it is observation from visual symptoms.

Senescence

The slower the process of leaf senescence, the better is the genotype. It is measured using scores on a scale from 0 to 10, dividing the percentage of estimated total leaf area that is dead. The description of this scoring is presented in Table 2.

Leaf rolling

The leaf rolling is a good indicator of dehydration avoidance mechanism. The best genotypes show the low scores. It is measured using scores from 1 to 5 (Table 3), when leaves are still more upright at the hottest time of the day.

Physiological traits

Canopy temperature

Canopy temperature measurements have been widely used to study the drought response of various crops. This approach is based upon the close, inverse relationship between leaf temperature and transpirational cooling. When stress develops and water status in low

Table 2. A scale of visual symptoms of leaf senescence.

Score	Description	Score	Description
1	10% dead leaf area	6	60% dead leaf area
2	20% dead leaf area	7	70% dead leaf area
3	30% dead leaf area	8	80% dead leaf area
4	40% dead leaf area	9	90% dead leaf area
5	50% dead leaf area	10	100% dead leaf area

Table 3. A scale of visual symptoms of leaf rolling.

Score	Description
1	Unrolled, turgid
2	Leaf rim starts to roll
3	Leaf has a the shape of a V
4	Rolled leaf rim covers part of leaf blade
5	Leaf is rolled like an onion

transpiration is reduced and canopy temperature rises. Blum et al. (1989) found a positive correlation between drought susceptibility of wheat genotypes and canopy temperature in stressed environments. Genotypes that suffered greater relative yield losses under drought stress tended to have warmer canopies at midday. Stark et al. (1991) also reported that the most drought-resistant potato genotypes usually had the lowest canopy temperatures during periods of drought. Canopy temperature is measured by a hand infrared thermometer (IRT) which is designed to sense long-wave infrared radiation emitted from its target and convert it to average temperature display which can be related to transpiration. Measurements (°C) will be made from 9:00 to 10:00 ideally on clear (cloudless), windless and sunny days.

Relative leaf water content (RWC)

Relative water content (RWC) is determined by weighing the entire leaves or leaf discs immediately after cutting, following prolonged immersion in water and after oven drying by the method described by Okono (2010). Three topmost fully expanded leaves were sampled from a plant. Six random replications were taken from a single plot. Each sample was represent a different plant within the genotype. The leaf sampling was taken in the afternoon after 2:00 pm, when the change in atmospheric condition and solar radiation slow in order to reduce change in water relation with time. Each sample was be placed in plastic bag. The plastic bag was immediately be placed in a cooler (at around 10-15°C). In the laboratory, each leaf in a plastic bag was weighed to obtain the leaf sample weight (fresh weight, FW), after which the sample was immediately placed hydrated to full turgidity for four hours under normal room light and temperature conditions. Samples were re-hydrated by floating on deionised water in a covered petri dish. After four hours, the samples were taken out of the water and will be thoroughly dried of any surface moisture quickly and lightly with paper towel and immediately weighed to obtain the fully turgid weight (TW). Samples are then oven dried at 80°C for 24 h and weighed (after being cooled down in a desiccator) to determine the dry weight (DW). All weighing was done to the nearest mg. The relative water content is calculated as followings:

$$\text{RWC } (\%) = \left(\frac{FW-DW}{TW-DW}\right) X\ 100\ ,$$

where FW: = sample fresh weight, TW: sample turgid weight; and DW: sample dry weight.

Stomatal conductance

Stomatal responses to environmental drought have a substantial influence on plant adaptation to arid climates (Bates and Hall, 1982). Stomata regulate water use and the development of water stress, and they influence plant growth rates through effects on carbon dioxide assimilation. Stomatal conductance is measured by using diffusion porometer. It can be calculated by the following empirical formula by Ball-Berry et al. (1987) model as modified by Xu and Baldocchi (2003):

$$g = g_0 + m\frac{ARH}{C_a}$$

Where, g is stomatal conductance, RH is the relative humidity at the leaf surface, C_a is the CO_2 concentration at the leaf surface, and g_0 and m are the intercept and slope, respectively.

Tuzet et al. (2003) suggested another model for determining stomatal conductance which incorporated the stomatal response to leaf water potential as follows:

$$g_{CO2} = g_0 + \frac{aA}{ci-\Gamma}.f\psi\nu$$

Where, g_{CO2} is the stomatal conductance for CO_2, g_o is the residual conductance, A is assimilation rate, ci is the CO_2 concentration in the intercellular spaces, Γ is the CO_2 compensation point and a is an empirical coefficient. Stomatal conductance varies with leaf irradiance, leaf temperature, atmospheric water vapour pressure deficit and CO_2 concentration (Tuzet et al., 2003). On the other hand, stomatal conductance depends on guard cell and epidermal turgor.

Cell membrane thermo-stability (CMS)

The CMS is highly correlated with yield under drought stress, therefore, this trait can assist to identify drought

tolerance in crops (Blum, 2005). Identification of cell membrane stability consists of measurement of electrolyte using the conductimetric method as described by Blum (2011). 15 to 20 cm^2 leaf materials are sampled from 5 plants of each genotype grown on control and drought stress conditions and placed in tubes. These leaves samples are washed three times with 20 ml desionised water. Clean samples are soaked in 20 ml of desionised water and incubated at 10°C for 24 h. The conductivity (T1) of the liquid of each tube is measured after 1 h of equilibration at room temperature. Each tube is covered and autoclaved for 15 min to kill the leaf tissues. The conductivity (T2) of each autoclaved sample is measured again after equilibration of one hour at room temperature. The CMS is determined with the following formula:

$$CMS\ (\%) = \frac{1-(1-T_1/T_2)}{1-C_1/C_2} \times 100$$

Where T1 and T2: Treatment conductivities before and after autoclaving, and C1 and C2 = control conductivities before and after autoclaving.

Leaf chlorophyll content

Strong correlations between higher chlorophyll and carotenoids contents and stress tolerance in plants were reported. It was observed that the chlorophyll content is different between drought resistant and susceptible genotypes. Drought resistant genotypes showed the highest chlorophyll content. This characteristic was suggested to be used as screening tool for drought tolerance (Ganji Arjenaki et al., 2012). Houman et al. (2011) reported that genotypes with high chlorophyll index under drought stress conditions revealed the highest yield. It was observed that drought stress had a negative effect on yields of different genotypes but the genotypes that were able to maintain their chlorophyll content were also able to maintain its yield potential (Khayatnezhad and Gholamin, 2012). Drought stress imposed during vegetative growth or anthesis significantly decreased chlorophyll a, chlorophyll b and total chlorophyll content. The results showed that mesophyll resistance is the basic determinate of rate of photosynthesis under drought stress conditions. Under drought conditions, the drought tolerant variety gave the highest yield whereas the drought sensitive variety gave the lowest yield (Mafakheri et al., 2010). Based on the above findings, the measurement of chlorophyll fluorescent was used as new tool to investigate effects of different stresses such as drought, salinity and heat on crop yield (Ort, 2002). Nowadays, handheld instruments such CCI-200 device and chlorophyll meter SPAD 502 Plus that measure the leaf chlorophyll content are available. These instruments determine the relative chlorophyll present in leaf by measuring the absorbance of leaf.

BIOTECHNOLOGICAL APPROACHES

Many biotechnological studies based on molecular biology have the aim to show the gene function and gene expression profile. To achieve this goal, different molecular approaches such as analysis of genome, transcriptome, metablome and proteome were developed (Carpentier, 2007). The analysis of the genome is a complex study of an organism's genome. This discipline known as genomics, is based on a complete genome analysis and involves DNA sequencing, assembly of sequences, annotation and mapping of genes (Arabidopsis, 2000).

The study of gene expression and its regulation is another approach for understanding the gene function. This approach known as transcriptomics uses efficient tools such as microarray analysis, cDNA fragment fingerprinting and serial analysis of gene expression (SAGE) to carry out the transcriptome analysis (Brown and Botstein, 1999; Schena et al., 1998). Metabolome represents a collection of all metabolites in a biological organism at a specific time and under specific conditions. These metabolites are the end products of genes expression of the organism.

The study of metablome (metabolomics) is a comprehensive, qualitative and quantitative study of all small molecules (less than or equal to 1500 daltons) participating in important metabolic functions and fulfilling critical roles such as drought tolerance, signaling molecules or secondary metabolites in an organism (Oliver et al., 1998). The main methods for metablome analysis are metabolite profiling and metabolite fingerprinting (Hall, 2006). Proteomics is another approach to understand the gene function and expression profile. It focuses on the characterisation of the cellular proteome which is defined as a set of protein species present in a biological unit at a specific developmental stage and under determined external biotic and abiotic conditions (Jorrín et al., 2006; Klug et al., 2000; Prescott et al., 2005). Proteomics involves protein biochemistry and bioinformatics to determine the spatial and temporal expression of proteins in cells and tissues of a living thing (Karr, 2007). The main tool of proteome analysis is a two dimensional gel electrophoresis (2-DE).

All these approaches (genomics, transcriptomics, metabolomics and proteomics) are powerful tools for massive screening of several genes and aim to reveal the changes of what might be happening in a cell. They can also serve as tools to identify candidates with desired traits such as drought tolerance in sweet potato germplasm.

CONCLUSION

The effect of drought stress on yield is highly complex and involves diverse processes in plants. Tolerance to a combination of different stress conditions, particularly those that mimic the field environment, should be the focus of future research programmes aimed at developing transgenic crops with an enhanced tolerance to naturally occurring environmental conditions. Modern genetic and molecular tools will significantly contribute to understanding physiological mechanisms related to drought and forge the way forward to develop new crop varieties with drought tolerance. The advent of biotechnology will contribute significantly to survey drought tolerance in various crop species, including sweet potato, cassava and potato. Further, advancements in marker technology and genetic transformation are expected to contribute significantly to the development of plants with various tolerances in the future. Integration of both agricultural practices, conventional and contemporary breeding strategies are necessary in developing crop varieties tolerant to abiotic and biotic stresses to insure food security of the worldwide population.

Conflict of Interests

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

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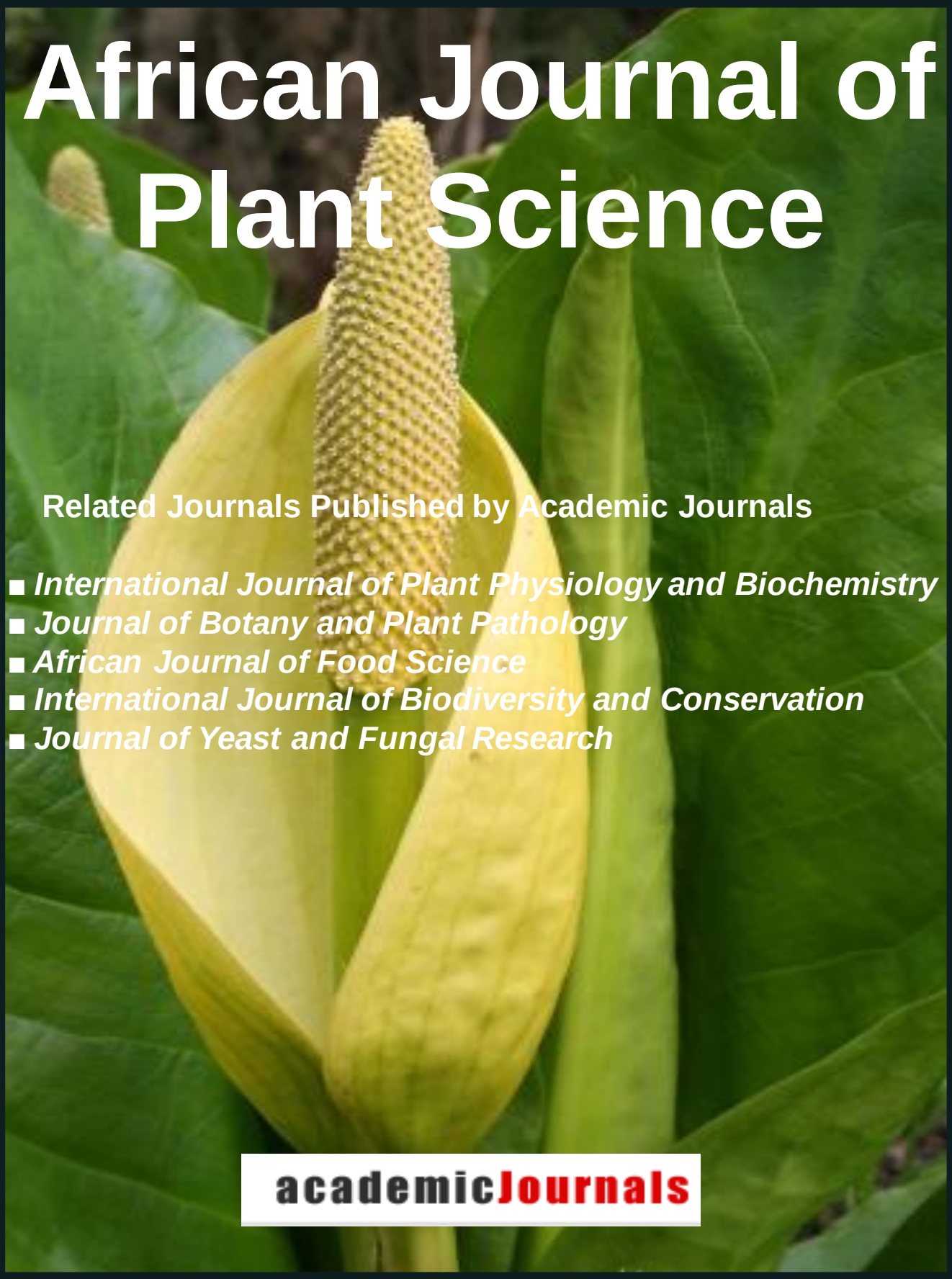

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