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Full Length Research Paper

Breeding for nutritional quality for *Corchorus olitorius*, *Annona muricata* and *Pentaclethra macrophylla* 1: A study of their antinutritional contents

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Breeding for nutritional quality of food/feed crops had somewhat been neglected by plant breeders all through the years. The objectives had mainly been focused on disease resistance and yield. Current concerns about the global food security need to encompass the issue of breeding for nutritional quality of food plants. One of the issues that revolve around the nutritional quality is the presence of antinutrients. These substances reduce the bioavailability of nutrients such as proteins, vitamins and minerals which could result in malnutrition especially in developing countries. Designing breeding programmes for the enhancement of nutritional quality of food crops require information on the types and concentrations of these antinutrients in such crops. This study was thus initiated to obtain information on the concentrations of the antinutrients - cyanogenic glycosides, oxalates, phytic acids, tannins and alkaloids - of *Annona muricata* (a fruit), *Corchorus olitorius* (a vegetable/grain crop) and *Pentaclethra macrophylla* (a legume) which are common among the local people of South East of Nigeria. Results showed that mean contents of oxalate, phytic acid, tannins and alkaloids were within permissible limits while high contents of cyanogenic glycosides were observed in *C. olitorius* (0.551 ± 0.0165 mg/100 g) and *P. macrophylla.* Fermentation did not reduce the level in *P. macrophylla.* The values obtained in unfermented and fermented seeds were 0.577 ± 0.0004 mg/100 g and 0.575 ± 0.003 mg/100 g respectively. The results were discussed bearing in mind the need for plant breeders and nutritionists to design programmes that will balance the adverse and beneficial effects of these antinutrients since many of them have also been found to be pharmacologically beneficial.

Key words: Antinutritional, contents, breeding, quality.

INTRODUCTION

The major aims of plant breeding since its early days had been on plant performance, disease resistance and yield (Raboy, 2013). Very few programmes had dwelt on nutritional quality. And even in recent times when concerns had mounted about the future of global food security, researchers and policy makers had routinely

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forgotton to mention the issue of nutritional quality (Raboy, 2013). The author, however, predicts that very soon "breeding programmes aimed at enhancing the nutritional quality and health-beneficial properties of staple food and feed crops may be central to and even critical to the sustainability of agriculture and food security world-wide".

One of the issues that revolve around the nutritional quality of food crops is the presence of antinutrients otherwise known as secondary metabolites. Antinutrients are compounds which sedentary organisms (plants, fungi and bacteria) synthesize to protect them from herbivores, insects, pathogens or adverse growing conditions. When plants containing these compounds, however, are eaten by domestic animals or humans, adverse physiological effects usually result (Khokhar and Apenten, 2003). Examples of these antinutrients which occur in varying degrees include, oxalate, phytate, cyanogenic glycosides, tannins, flavonoids, saponins and alkaloids (Soetan, 2008; Musa et al., 2011).

These substances cause a reduction in the bioavailability of nutrients like proteins, vitamins and minerals. Consequently even when these nutrients are present in foods, the body does not fully make use of them (Kolawole and Obueh, 2013) thus resulting in malnutrition especially in developing countries. This effect occurs because of the interaction of these antinutrients with normal nutrients. Phytates and oxalates, for example, are strong chelators that react with minerals such as calcium, magnesium, zinc, copper, iron etc to form complexes that cannot be absorbed by the intestine (Akande et al., 2010). Such complexes are excreted thus reducing the bioavailability of the minerals to the body. Kidney stones are formed as a result of such complex formation between calcium and oxalate (Ogbadoyi et al., 2011). The phytates and oxalates also affect protein digestion.

Cyanogenic glycosides are hydrolysed by enzymes to give off hydrogen cyanide (HCN) which is a potent respiratory poison. The HCN is associated with many of the diseases of the central nervous system and it is very toxic even at low concentrations (Akande et al., 2010; Kolawole and Obueh, 2013). Tannins and Saponins can interfere with protein digestion (Dei et al., 2007) while alkaloids cause gastrointestinal and neurological disorders (Aletor, 1993). Reports showed that some plant alkaloids can cause infertility (Olayemi, 2007).

Several authors have noted that these antinutrients are usually reduced or eliminated through various processing techniques such as cooking, drying, blanching etc. (Akwaowo et al., 2000; Akinyeye et al., 2011). Unfortunately, these techniques cause concomitant decrease in protein, fat, ash, various vitamins and minerals contents of those plants (Shokunbi et al., 2011; Ogbadoyi et al., 2011).

According to Akande et al. (2010) for one to authenticate the nutritional potential of a plant, information on the type, nature and concentration of antinutrients present in that particular plant should be obtained. This assertion is corroborated by the report of Nwanjo et al. (2006) who concluded from their experiments that *P. macrophylla* is nutritional but its nutritive value is slightly hindered by the presence of antinutritional factors present in the seeds. The information thus obtained on the concentrations of the antinutrients will be valuable to plant breeders for designing breeding programmes. Breeding genotypes with low levels of these antinutrients is the best option in the long run for solving the problem of antinutrients. Lott et al. (2011) for example advocated for the breeding of crop genotypes with low levels of phytate. According to the authors, this will contribute to a global effort to enhance the appropriate utilization of phosphorus in agricultural production.

This research was, therefore, undertaken to assess some of the antinutrients present in the seeds of three nutritious plants (Okeke et al., 2008) that are common among the local people of the South East region of Nigeria. The species have been listed as part of the underutilized crop species in Africa. These are: (1) *Annona muricata* (Soursop, family Annonaceae). Compositional analyses have shown that the seeds contain 21.43 to 27.34% protein (Awan et al., 1980; Fasakin et al., 2008), carbohydrate (4.36%), fat (22.57%), magnesium (53.3%), iron (63.2%) and so on (Fasakin et al., 2008). The seeds along with the bark, leaves and stem of this species are very popular components of local preparations for handling cancer cases. They contain chemicals known as Annonaceous acetogenins. These chemicals are found only in the Annonaceae family and they have been reported to have antitumorous, antiparasitic, insecticidal, and antimicrobial activities. Numerous studies have shown that they selectively inhibit enzymes present only in the membranes of cancerous cells and thus they are toxic to cancer cells and non-toxic to normal cells (Morton, 1987; Oberlies et al., 1995; Gupta et al., 2011; Gajalakshmi et al., 2012). The seeds and roots, however, have been found to possess some alkaloids that have shown some neurotoxic effects. (2) *Corchorus olitorius* (Bush Okro, family Sparrmaniaceae). It has abundant levels of β-carotene, iron, calcium, vitamin C. It has been listed as one of the seven highly valued indigenous leafy vegetables in Nigeria (Adebooye et al., 2003) and one of one hundred orphan crops whose genome will soon be sequenced, assembled and annotated (Mars Incorporated, 2013). Its seeds are used medicinally as a purgative (Gupta et al., 2003) and they also have a broad spectrum of antibacterial activity (Pal et al., 2006). (3) *Pentaclethra macrophylla* (African oil bean, Family, Fabaceae) popular for its oil-rich seeds. The seeds, however, are inedible when raw and very bitter until the final stage of fermentation. They have been reported to contain high quantity protein (9.31%) and the 20 amino

acids (Achinewhu, 1982; Ikhuoria et al., 2008). One of the plants used in this study had earlier been listed by IPGR, 2002 as among the neglected and underutilized crop species of Africa so as much information as possible are needed on them to guide their improvement.

MATERIALS AND METHODS

The seeds of *P. macrophylla* and the fruits of *A. muricata* were sourced from Nsukka town in Enugu State of Nigeria while the seeds of *C. olitorius* were obtained from National Centre for Genetic Resources and Biotechnology (NACGRAB) Moor Plantation, Ibadan, Nigeria. The samples were authenticated by Mr Alfred Ozioko of Biodiversity and Conservation programme/International Centre for Ethnomedicine and Drug Development located at No. 110 Aku Road Nsukka. The seeds of *A. muricata* were separated from the flesh and oven-dried. The method of Amadi et al. (2011) was used to induce fermentation for 2 days in some of the seeds of *P. macrophylla*. Cooked dehulled and oven-dried samples were used as the unfermented sample. All the respective seeds were separately ground into powder with the aid of a blender. For assessing the levels of oxalate and alkaloid, one gram (1 g) of each powder was used respectively while 0.5 g was used for cyanogenic glycosides, phytate and tannins. Pearson's (1976) methods were used for all the assessments. The procedures were as follows:

1. Determination of phytic acid (Pearson 1976):

a. 0.5 g of the sample was weighed into a test tube.
b. This was macerated with 20mls of 1.2% Hydrogen Chloride + 10% Sodium sulphate.
c. The tube was shaken vigorously for 10 min and allowed to stand for 2 h with intermittent shaking every 10 min.
d. The solution was subsequently filtered.
e. 5 ml of the filtrate were transferred into triplicate tubes.
f. 5 ml of water and 6 ml of 2 g ferric chloride + 17 ml hydrogen chloride per litre were added and these were:

i. Mixed and boiled for 75 min in a water bath.
ii. Cooled for one hour at room temperature
iii. Centrifuged for 15 min at 3000 r.p.m.
iv. The supernatant was decanted and
v. The residue was washed with 0.6 hydrogen chloride + 2.5% sodium sulphate.
vi. The residue after washing was centrifuged again and decanted.
Vii. To the residue, 5 ml of concentrated Nitric acid and 4 ml of concentrated sulphuric were added and
viii. Transferred to a girdal flask.
ix. The mixture was heated on a hot plate for 30 min until only the sulphuric acid remained.
x. It was allowed to cool after which 5 drops of Hydrogen peroxide were added and the mixture heated for further 10 min.
xi. It was cooled and 3 ml of normal hydrogen chloride were added and the mixture was heated for 5 min.
xii. It was neutralized with 5 normal sodium hydroxide and made up to 10 ml with water and the phosphorus content determined.

2. Determination of phosphorous (Pearson, 1976):

a. 5 ml of the digest was transferred into triplicate tubes.
b. 2 drops of nitric acid and 2.5 vanadate molidate reagent was added.
c. These were mixed and 2.5 ml of water added and
d. The absorbance was taken at 470 nm against a blank.

3. Determination of oxalate (Pearson, 1976):

a. 1 g of the sample was weighed and put in a test tube.
b. 47.5 ml of water and 2.5 ml of 6 normal hydrogen chloride were added to the tube.
c. The mixture was boiled for an hour and made up to 62.5 ml with water.
d. The tube was cooled at room temperature and the contents subsequently filtered.
e. 12.5 ml of the filtrate was taken and the pH was adjusted to fall between 4.0-4.5 with dilute ammonia (NH_3).
f. This was heated up to 90°C and subsequently filtered.
g. The mixture was heated again up to 90°C
h. 5 ml of calcium chloride was added with constant stirring.
i. The tube was allowed to stand overnight.
j. Centrifuged for 5 min and the supernatant decanted off.
k. The precipitate was dissolved with 5 ml of 20% sulphuric acid.
l. And the mixture heated until about to boil.
m. This was titrated with 0.05 normal standard potassium permanganate ($KMnO_4$) until pale pink colour that persists for 30 s.

4. Determination of alkaloid (Pearson, 1976):

a. 1 g of the sample was weighed and put in a test tube and macerated with 10 ml of 20% sulphuric acid and10 ml of ethanol for 10 min. The tube was allowed to stand for an hour with intermittent shaking and subsequently centrifuged for 5 min.
b. 0.5 ml of the supernatant was transferred in triplicate tubes.
c. 2.5 ml s of 60% sulphuric acid was added and the two were mixed.
d. 2.5 ml of 0.5% formaldehyde was subsequently added and the test tubes were allowed to stand for 3 h.
e. The absorbance was taken at 565 nm against a blank.

5. Determination of cyanogenic glycoside (Pearson, 1976):

a. 0.5 g of the sample was weighed and put in a test tube.
b. The sample was macerated with 20 ml of phosphate buffer pH 6 for 10 min.
c. The test tube was allowed to stand for an hour with shaking every 10 min interval.
d. It was later centrifuged for 5 min.
e. 1 ml of the supernatant was transferred into triplicate tubes.
f. 4 ml of alkaline picrate was added and boiled for 5 min in a water bath.
g. The tube was cooled in cold water and the absorbance was taken at 470 nm against a reagent blank.

6. Determination of tannin (Pearson, 1976):

a. 0.5 g of the sample was weighed and put in a test tube.
b. The sample was macerated with 20 ml of methanol for 10 min and centrifuged for 5 min at 3000 r.p.m.
c. 5 ml of the supernatant was transferred into triplicate tubes.
d. 0.3 ml of 0.1 M ferric chloride in 0.1 M hydrogen chloride were added.
e. The solution was mixed.
f. 0.3 ml of 0.0008 M potassium ferricyanide was added.
g. The solution was mixed.
h. The absorbance was taken after 5 min at 720 nm against a blank.

The design of the experiments is as shown in Table 1. The means of the antinutrients for each species were calculated and subsequently used to plot a bar chart to depict graphically the contents of the antinutrients in each of the species.

Table 1. Design of experiments – randomised complete block design with three replications for each antinutrient per species was employed.

Antinutrients/species	*A. muricata*	*C. olitorius*	*P. macrophylla*	
			Unfermented	fermented
Cyanogenic glycosides	1	1	1	1
	2	2	2	2
	3	3	3	3
Oxalate	1	1	1	1
	2	2	2	2
	3	3	3	3
Phytic acids	1	1	1	1
	2	2	2	2
	3	3	3	3
Tannins	1	1	1	1
	2	2	2	2
	3	3	3	3
Alkaloids	1	1	1	1
	2	2	2	2
	3	3	3	3

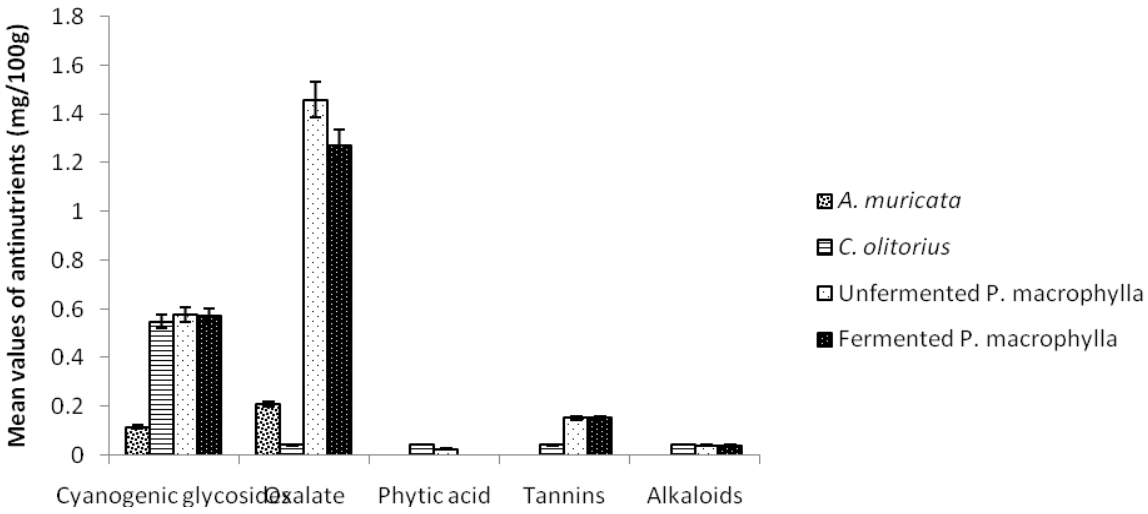

Figure 1. Comparison of the antinutrients of the species.

RESULTS AND DISCUSSION

All the antinutrients were found in the species but at varying levels. A combined comparison of the antinutrients for all the species is depicted in Figure 1. Oxalate was found to be the highest antinutrient and this high content was found in unfermented and fermented seeds of *P. macrophylla*. The content in unfermented seeds was, however, higher than that of fermented seeds. The presence of cyanide, oxalate and saponins in *P. macrophylla* were also observed by Onwuliri et al. (2004).

The minimum lethal dose of cyanogenic glycoside orally taken by man has been reported to be between 0.5 mg and 3.5 mg/kg body weight (Wobeto et al., 2007; Fowomola, 2010) while the lethal dose for cattle and sheep is 2.0 to 4.0 mg per kg body weight (Kumar, 1991). The concentrations obtained in this study were 0.551 ± 0.0165 mg/100 g for *C. olitorius*, 0.577 ± 0.0004 mg/100 g for unfermented seeds of *P. macrophylla*, 0.575 ± 0.003

mg/100 g for fermented seeds of *P. macrophylla* and 0.1183 ± 0.3687 mg/100 g for *A. muricata*. Thus *C. olitorius* and *P. macrophylla* had high concentrations of cyanogenic glycosides which were above the acceptable limits. Fermentation did not really reduce the concentration in *P. macrophylla*. This is not in consonance with the report of Amadi et al. (2011) who observed a reduction in the concentrations of phytates, tannins, saponins and complete elimination of flavonoids, alkaloids and cyanogenic glycosides in *P. macrophylla* following fermentation. The antinutrient, cyanogenic glycosides have been linked to many of the diseases associated with the central nervous system (Kolawole and Obueh, 2013).

Reports have shown that the lethal dose of oxalate is between 200 and 500 mg/100 g (Pearson, 1976). Noonan and Savage (1999) noted that the intake of 4 to 5 g of oxalate is the minimum dose that can result to death in an adult human. They further reported that a number of authors had showed that 10 to 15 g could be lethal. The levels obtained in this study were within acceptable limits. They ranged from 0.044 ± 0.0443 to 1.460 ± 0.002 mg/100 g.

The tannin content of the species studied ranged from 0.0039 ± 0.0004 to 0.155 ± 0.002 mg/100 g. These values were very low when compared with values reported by several researchers. Aletor and Adeogun (1995) reported that high level of tannins (76 to 90g kg DM^{-1}) could be very lethal if consumed. Sheep that consumed 0.9 g hydrolysable tannins kg/body weight showed signs of toxicity in 15 days (Kumar, 1991).

Large amounts of phytic acids have been reported to be present in fiber-rich foods. Such food, however, are pharmacologically recommended because they protect humans from cardio vascular diseases and some forms of cancer (Ensminger and Ensminger, 1996; Norhaizan and Nor-Faizadatul-A, 2009). Inspite of this advantage, phytic acid reduce bioavailability of minerals because it has strong binding affinity to them. This chelation process increases the incidence of mineral deficiency diseases because the minerals are made unavailable for absorption by the intestine (Ekholm et al., 2003). Fortunately, the phytic acid contents observed in this study were quite low.

From this study, it can be concluded that cyanogenic glycosides are the antinutrients to consider while designing programmes for the improvement of *C. olitorius* and *P. macrophylla*. Some authors (Khokhar and Apenten, 2003; Soetan, 2008), however, made some observations concerning the complete removal of antinutrients through classical breeding or through biotechnological techniques. They noted that:

1. Since the antinutrients are critical for the survival of the plants that harbor them, complete removal may lead to reduction in growth of the plants as well as reduction in yield.
2. Complete removal will also eliminate the pharmacological and medicinal properties of these compounds. Many of them have been reported to possess anticarcinogenic activity (for example, phytates, saponins, phenolic acids etc), antimicrobial activity (saponins, flavonoids, tannins), anthelmintic activity (tannins, saponins), hypocholesterolaemic activity (saponins) and pharmacological applications (tannins, saponins and flavonoids are constituents of several drugs).

The authors, therefore, suggested that both the adverse and beneficial properties of the compounds should be borne in mind by both the plant breeders and nutritionists while designing programmes for the improvement of the quality of the species.

Conflict of Interests

The authors wish to declare that there are no conflicts of interests in this work.

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Structure-related properties of sweetpotato critically impact the textural quality of fried chips

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This study sought to define what attributes of sweetpotatoes are most critical to textural qualities of their fried chips for effective selection of specialty cultivars. It compared texture-predicting fracturability of fried chips prepared from either structurally intact or disrupted slices of 13 cultivars; analyzed major attributes of these sweetpotatoes, including starch contents and properties, dry matter contents, and structure-related penetration resistances (measured using an adapted penetration test); and evaluated correlational relationships between these attributes of sweetpotatoes and fracturability of fried chips. The study found that lower dry matter (<22.6% F.W.) and starch contents (<10% F.W.), and lower gelatinization temperatures of starch in sweetpotatoes generally resulted in a more favorable fracturability (lower peak break force) of fried chips. However, contrary to potato, total dry matter content is not the sole determinant of textural qualities of fried sweetpotato chips; instead, structure-related attributes of sweetpotatoes appear to have a greater impact. Partial structural disruptions of sweetpotato slices by blanching effectively improved fracturability of fried chips in all analyzed cultivars, and by ~40% in eight of the 13 cultivars. Furthermore, the degree of structural penetrability of sweetpotatoes, as indexed by penetration resistances, showed very significant correlations with fracturability of fried chips.

Key words: Sweetpotato chips, fracturability, puncture test, structure-related attributes, dry matter content, starch.

INTRODUCTION

Sweetpotato [*Ipomoea batatas* (L.) Lam.] is the seventh largest food crop in world production (FAOSTAT, 2010). It has many favorable agronomic attributes such as a short production cycle, tolerance of low soil fertility, and

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resistance to many production stresses (Kays, 2005; Ravi and Indira, 1998). Furthermore, sweetpotato storage roots offer excellent nutrition and health benefits due to the presence of abundant vitamins (Vitamin A precursor β-Carotene, Vitamin B1 and C, and one of the few non-fat sources of Vitamin E), antioxidant micronutrients, beneficial complex carbohydrates, minerals and dietary fibers (Bovell-Benjamin, 2007; Woolfe, 1993). Despite such benefits, utilization of sweetpotatoes for food and industrial raw materials has steadily declined in recent years, and this is mirrored by reduction of its annual worldwide production from a peak of ~1.5×10^8 t in 1978 to ~1.06×10^8 t in 2006 and thereafter (FAOSTAT, 1961 to 2010).

Although annual production of sweetpotatoes in the U.S. rose from ~6.25×10^5 t in 2000 to ~1.08×10^6 t in 2010 (FAOSTAT), it is still a greatly underutilized and undervalued crop. The average annual per capita consumption of sweetpotatoes from 2000 to 2010 in the U.S. was a meager ~2.1 kg, about 30 times lower than the annual ~58.0 kg per capita consumption of potato (2010 Vegetable and Melon Year book, ERS-USDA). This low consumption, which both reflects and results in low demand and low commercial value for sweetpotatoes, can be largely attributed to its poor utilization in popular food products that have mass-market appeal, and its lack of use in large industrial applications. Recent efforts to increase sweetpotato consumption and commercial value have led to increased utilization of sweetpotatoes or their processed products, including flour, puree, and frozen cooked products, for making popular food items such as bread with up to 65% sweetpotato flour, ready-to-eat breakfast cereals, bread puddings, casseroles, tarts, muffins, pancake mixes, snack food items (chips, french-fries and flakes) and beverages (Bovell-Benjamin, 2007; Padmaja, 2009). Some of these products, in particular, sweetpotato chips and fries, are now gaining popularity, and are being commercially produced by several large U.S. companies, including the Terra Chip brand from the Hain Celestial Group Inc. of Colorado, the Seneca Foods Corporation of New York, Utz Quality Food of Pennsylvania, the Pringles brand from Proctor and Gamble, the Route 11 Potato Chips of Virginia, Zapp's Potato Chips of Louisiana and Lamb Weston of Washington. However, sweetpotato chips and fries made from common cultivars were viewed to have less satisfactory taste and texture relative to similar products made from potato (Collins, 1993; Woolfe, 1993).

The common U.S. sweetpotato cultivars that were developed primarily for fresh markets are not entirely suitable for the manufacture of chips and fries, despite recent improvement of production technology (Da Silva and Moreira, 2008). Selection of novel specialty sweetpotato cultivars for making chips or fries with better textural qualities may help to sustain or enlarge their market shares.To define what attributes of sweetpotatoes are most critical to textural qualities of their fried chips for effective selection of such specialty cultivars, we investigated impacts of several major attributes of sweetpotatoes on texture-predicting fracturability (Bourne, 2002; Segnini et al., 1999a, b) of their fried chips.

MATERIALS AND METHODS

Plant materials

The sweetpotato breeding and genetics program of North Carolina State University kindly provided sweetpotatoes of 10 cultivars (Covington, Carolina Rose, Hernandez, Porto Rico, Diane, O' Henry, Hannah, NC-Japanese, Hi-Dry and Suwon-122) from the 2007 growing season, and their dry matter contents. Two additional cultivars (Beauregard and Jewel) examined in 2007, all the twelve cultivars in 2008, and the twelve cultivars plus Zhenghong-3 (Accession PI 606266) for evaluation of chip textures and all other cultivars for penetration tests in 2010 were grown under recommended conditions (Mississippi State University Extension Service,http://msucares.com/lawn/garden/vegetables/list/sweetpotato.html) at the Experimental Farm of Alcorn State University, Mississippi. The seeding plants of Zhenghong-3 and all other cultivars (excluding those cultivars from North Carolina State University) were initially obtained from the USDA-ARS Plant Genetic Resources Conservation Unit, Griffin, GA. All sweetpotatoes used in the study were not cured, and were not over one month in storage.

Measurement of the dry matter content

The dry matter content of sweetpotatoes was measured as completely dried weight per 100 g of slices randomly sampled out of 1 kg of sliced whole sweetpotatoes of representative sizes in triplicate, according to the standard method (Rodriguez, 1999). The reported dry matter contents of sweetpotatoes from the 10 cultivars that were initially provided by the North Carolina State University in 2007 are averages of the data from samples of the 2007 North Carolina and the 2010 Mississippi crops. The dry matter contents of the Zhenghong-3, Beauregard and Jewel sweetpotatoes are averages of triplicate sample measurements of the 2010 Mississippi crop.

Measurements of the total starch content

Fresh sweetpotato slices of 50 g randomly taken out of 1 kg slices of several whole sweetpotatoes of representative sizes were freeze-dried, ground using a coffee grinder and sieved to obtain fine dry powders (< 0.5 mm). The total starch content of a 100 mg dry sweetpotato powder sample was measured using a total starch assay kit (Megazyme International Ireland Ltd, Ireland), and normalized on a fresh-weight basis. The procedure recommended for samples containing resistant starch and soluble sugar was followed for all measurements. Triplicate measurements for all cultivars except Zhenghong-3 were made from two samples from the 2007 crop, and one from the 2008 crop. Measurements for the Zhenghong-3 sweetpotatoes were made with three samples from the 2010 crop.

Purification of starch and analyses of starch properties

About ~20 g sweetpotato slices randomly taken out of 1 kg slices of

several whole sweetpotatoes of representative sizes were further chopped and finely ground in cold water (1 ml H_2O per gram of tissue) using a Warring® Chopper/Grinder. Starch in the slurry was filtered through two layers of Miracloth into a 250 ml beaker, and allowed to settle for 30 min or so. The settled starch was recovered, re-suspended in ~30 ml of cold water, and transferred to a centrifuge tube. Starch was pelleted by centrifugation for 5 min at 6,000 g. Gel-like materials overlaying the packed white starch granules were scrapped using a spatula. Starch was then washed twice in a cold buffer (62.5 mM Tris-HCl, pH 6.8; 10 mM EDTA and 40 g kg^{-1} SDS), three times in cold water, and once in cold acetone (~15 ml) by repeated re-suspension and centrifugation. The purified starch was air dried, and stored in a sealed tube at -20°C. The purified starch samples of 20 to 25 mg (measured to the nearest 0.1 mg) were used for analyses of the amylose content using the Megazyme Amylose/Amylopectin assay kit. Multiple measurements (≥ 3) for all cultivars except Zhenghong-3 were made from independent samples from the 2007 and 2008 crops. Measurements for Zhenghong-3 were made from three samples from the 2010 crop.The gelatinization and retrogradation temperatures of all starch samples were determined by standard Differential Scanning Calorimetry (DSC).

Preparation of sweetpotato chips

Sweetpotatoes of 8.0 to 8.5 cm in diameter selected from each cultivar were lightly peeled, and had narrower ends cut off for size uniformity. The peeled and trimmed sweetpotatoes were sliced transversely into round pieces of ~1 mm in thickness using a Chef's Choice 632 slicer with a serrated blade. About 100 or 200 slices of diameters 8.0 to 8.5 cm were randomly sampled out of ~1 kg slices for each cultivar, and processed for texture measurements. Half of the sweetpotato slices from each cultivar were washed and soaked in ice water for at least 1 h, blot-dried and fried for chips as the unblanched control. The other half of the slices were soaked in ice water briefly, drained, blanched in slightly acidic and salty boiling water (~50 ml l^{-1} white vinegar and 4 g l^{-1} table salts) for 3 min, snap-chilled in ice water for 5 min or so, dried on racks in a drying oven for 1 h at 30°C and at room temperature for another 3 h, flattened and frozen at -20°C until ready for frying. Both blanched and unblanched slices were fried in Canola oil at 190°C for 1 min, which were experimentally optimized for the best fracturability of chips from all the 13 cultivars.

During initial optimization, both blanched and unblanched slices from 23 cultivars or breeding lines were fried at two oil temperatures, 176 and 190°C, to the extent of obvious partial burning (blackened, <1% moisture by dry matter measurement). The peak break forces of these partially burned chips were measured, and regarded as the lowest (that is, the best or maximal fracturability) for the type of chips under the maximal frying time. Six of these cultivars whose slices needed shortest or longest frying times (~40 s or ~150 s at 190°C) to reach partial burning were left out from further optimization and testing. Shorter frying times that would not significantly drop the best fracturability of chips were then sought for blanched and unblanched slices from some of the 17 cultivars or lines by frying in 190°C oil at 15-s decremental time points from their maximal frying time.The condition of frying in 190°C oil for 1 min was found to yield chips having the best fracturability for both types of slices of 12 cultivars. But, it also resulted in certain degree of burning to chips from some of these cultivars (especially those having lower dry matter contents). This frying condition was adopted for preparing chips from these 12 cultivars, plus Zhenghong-3 at a later time for comparison.

Fracture, puncture and penetration tests

The fracture test for objective evaluation of textural properties of sweetpotato chips were performed essentially following the method of Segnini et al. (1999a, b), modified by the use of a custom-made cylinder holder (~19.05 mm diameter) instead of a three-point support to overcome the surface-curling problem of the fried chips. A TMS-Pro texture analyzer (Food Technology Corporation, Virginia) equipped with a 100 N calibrated load cell, a 12.7 mm ball probe and the custom-made cylinder holder (~19.05 mm diameter) was used for the test. A sweetpotato chip was placed on top of the cylinder holder with a nearly complete circular touch on the holder, and subjected to fracturing by the downward travelling ball probe. The ball probe was programmed to travel at a constant speed of 15 mm per min to a target displacement of 1 mm from where the load cell records a 0.5 N load. The peak load (that is, the maximum force of break) was quantified using a load versus displacement graph.

The penetration tests were performed using the TMS-Pro texture analyzer equipped with a 100 N calibrated load cell, TMS 3 mm diameter cylinder probe and a standard fixture table. Sweetpotatoes were either halved along the longitudinal root axis, or had both ends cut off transversely. Halved sweetpotatoes were placed on the fixture table with either the radial face down or up for the probe to puncture through the peripheral or radial face. The end-trimmed sweetpotato chunk was placed with one transverse face down on the fixture table for the probe to puncture parallel to the longitudinal root axis through the transverse face. The cylinder probe was programmed to travel at a constant speed of 250 mm per min to a target penetration displacement of 10 mm from where the load cell records a 0.5 N load. The average load for each 10 mm penetration was calculated from the load versus displacement graph.At least 50 individual penetrations of about 2 to 3 mm apart were made on each face for calculating the mean penetration resistance. The cylinder probe is small enough so that it only punctures a hole of about 3 mm in diameter on the testing surface, and causes no disruption of tissues 2 to 3 mm away from the hole.

Statistical analyses

The Prism 5 (GraphPad Software, La Jolla, CA) statistics and graphing software package was used for all statistical analyses.

Preparation of microscopy slides

Standard microtomy and microscopy procedures (Ruzin, 1999) were followed for preparation of paraffin-embedded sections of tissues (transverse and tangential sections), which were taken from blanched or unblanched sweetpotato slices, and for staining the sections with Toluidine Blue.

RESULTS

Instrumental evaluation of the textural quality of sweetpotato chips

The textural quality of fried sweetpotato chips was objectively evaluated via their mechanical fracturability, which was measured as the peak fracture force. As illustrated by load versus displacement curves from fracturing four sweetpotato chips (Figure 1), the peak loads registered the maximal forces of break, which were also the forces that caused the first major fractures characterized by a minimum of 10% load drop. These peak loads thus measure both hardness and brittleness of the chips. The peak loads have been demonstrated to

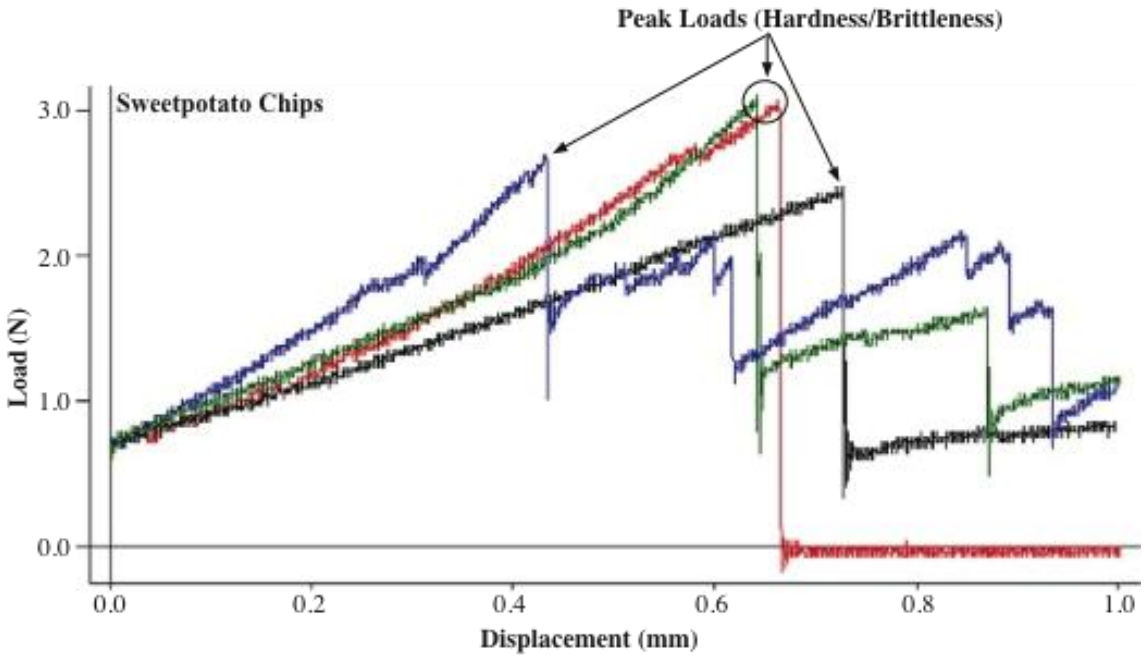

Figure 1. Illustration of typical load-displacement curves from fracture tests of fried sweetpotato chips. A sweetpotato chip was placed on top of a customer-made cylinder holder with a nearly complete circular touch on the holder, and subjected to fracturing by the downward travelling ball probe. The ball probe was programmed to travel at a constant speed of 15 mm per min to a target displacement of 1 mm from where the load cell records a 0.5 N load. Four load curves (in different colors) from fracture tests of 4 fried chips made from blanched slices of Covington sweetpotatoes are shown. The peak fracture loads registering the maximal forces of break and the forces causing the first major fracture marked by a 10% load drop measure hardness and brittleness of the chips.

correlate tightly with (so as to predict) the sensorial textures of tenderness and crunchiness (Segnini et al., 1999a). The peak fracture force, which is the average of peak loads of 30 or more representative chips, should thus predict tenderness and crunchiness of sweetpotato chips. In other words, a larger peak fracture force indicating less favorable fracturability should predict less sensorial tenderness and crunchiness. All the chips from the 13 selected cultivars were fried to reach lowest levels of their respective peak fracture forces (or maximal fracturability, indicating maximally fried) so that residual moisture contents of these fried chips should have no impact on the comparison of chip fracturability. Since a uniform frying condition was used for all chips for the purpose of simplifying the comparison, chips from some of the cultivars (especially those with lower dry matter contents) did incur various degree of burning.

Impacts of sweetpotato structure-related attributes on the textural quality of chips

The impact of the storage-root tissue structure on the textural quality of fried sweetpotato chips was first assessed by comparing the fracturability of chips made from slices with or without structural disruption. Limited structural disruption was achieved mainly by blanching sweetpotato slices in acidic water with added salts. Microscopic analyses revealed that this treatment resulted in starch gelatinization and partially collapsed or broken cell walls in a cross-section of a blanched slice compared to a control slice, as well as separation or disruption of xylem elements in a tangential section of a blanched slice (Figure 2A). Furthermore, blanching under slightly acidic and salty conditions appeared to help fix the natural colors of sweetpotato slices. Blanched slices from all 13 cultivars, as compared with unblanched controls, yielded chips with a more favorable fracturability, and generally better appearance and less apparent retention of oil on the surface, as exemplified by those from the cultivar Hernandez in Figure 2B. As summarized in Figure 3, the peak fracture forces of blanched chips from the 13 cultivars range from 1.96 ± 0.48 N for Zhenghong-3's to 4.59 ± 0.84 N for Suwon-122's, and those of unblanched chips range from 3.62 ± 0.86 N for Zhenghong-3's to 5.12 ± 0.82 N for Suwon-122's. The peak fracture forces of all these sweetpotato chips except that of blanched chips from Zhenghong-3 are much larger than those of a commercial brand of potato chips ranging from 0.75 to 2.00 N, which predicts a much less favorable textural qualities of sweetpotato chips. The limited structural disruption by blanching

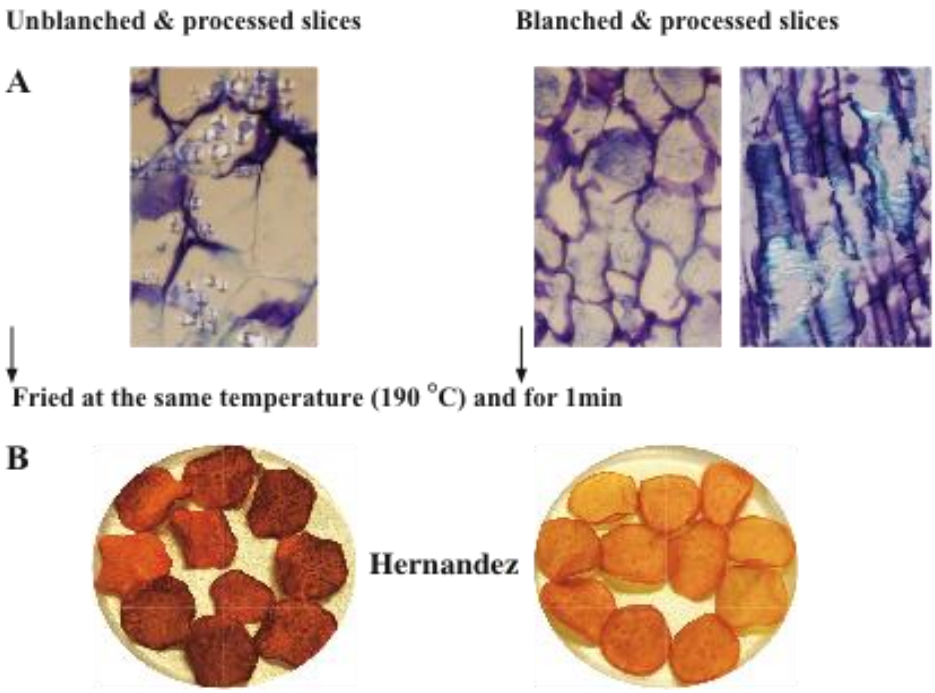

Figure 2. Illustration of the partial structural disruption in blanched sweetpotato slices and its effect on fried chips. To partially disrupt tissue structure, sweetpotato slices were blanched in slightly acidic and salty boiling water, snap-chilled, slowly dried, flattened and frozen before frying. The blanched and control slices were fried in oil at the same temperature (190°C) for 1 min. (A) As compared with intact storage parenchyma cells on a cross section of an unblanched slice (left), corrupted or detached cell walls of storage parenchyma cells are evident on a cross section of a blanched slice (middle). In addition, corrupted or broken xylem elements can be seen on a tangential section of a blanched slice (right). The sections were stained with Toluidine Blue. (B) The lower panels shows appearances of representative fried chips made from unblanched and blanched slices of Hernandez sweetpotatoes.

reduced the peak fracture forces, or increased fracturability, of chips by an average of ~40% for eight cultivars (41.1% for Hernandez, 40.7% for Beauregard, 38.1% for Carolina Rose, 41.2% for Diane, 39.1% for Jewel, 43.0% for O' Henry, 39.5% for Hannah and 45.9% for Zhenghong-3), by an average of ~25% for three cultivars (26.1% for Covington, 25.5% for Porto Rico and 21.5% for NC-Japanese), and to a lesser extent for two cultivars (16.7% for Hi-dry and 10.4% for Suwon-122).Sweetpotatoes of the latter two cultivars both have high dry matter and starch contents relative to the other cultivars. The limited structural disruption could even bring the fracturability of blanched chips from Zhenghong-3 to a level that is comparable to that of commercial potato chips (1.96 ± 0.48 N versus 0.75 to 2.00 N peak fracture forces). This effective improvement of fracturability in blanched chips by limited structural disruption indicate that certain unfavorable structural attributes of sweetpotatoes are responsible for ~25 to 40% loss of fracturability, and thus loss of similar degrees of sensorial tenderness and crunchiness, in unblanched chips from 11 out of the 13 evaluated cultivars.

To further assess the impact of structural attributes of sweetpotatoes on textural qualities of fried chips, we attempted to quantify structural mechanical properties related to hardness, brittleness and firmness of sweetpotato tissues using an adapted puncture and penetration test. This test measures the tissue resistances against penetrations of a cylindrical probe over a target distance (10 mm) through the peripheral and radial faces of a longitudinal half of a sweetpotato, and through the transverse face of a transversely cut section to register the structural mechanical properties of a sweetpotato in both tangential and longitudinal directions.

As illustrated by the load versus displacement curves comparing the tests through peripheral faces of sweetpotatoes and potatoes from three and two cultivars, respectively (Figure 4A), clear distinctions of structural mechanical properties can be noted among the different types of sweetpotatoes, as well as between sweetpotatoes and potatoes. The average load over a 10 mm penetration calculated from the curve represents the local resistance along the penetration path. The average load from multiple puncture and penetration tests of a single sweetpotato sample or many sweetpotato samples

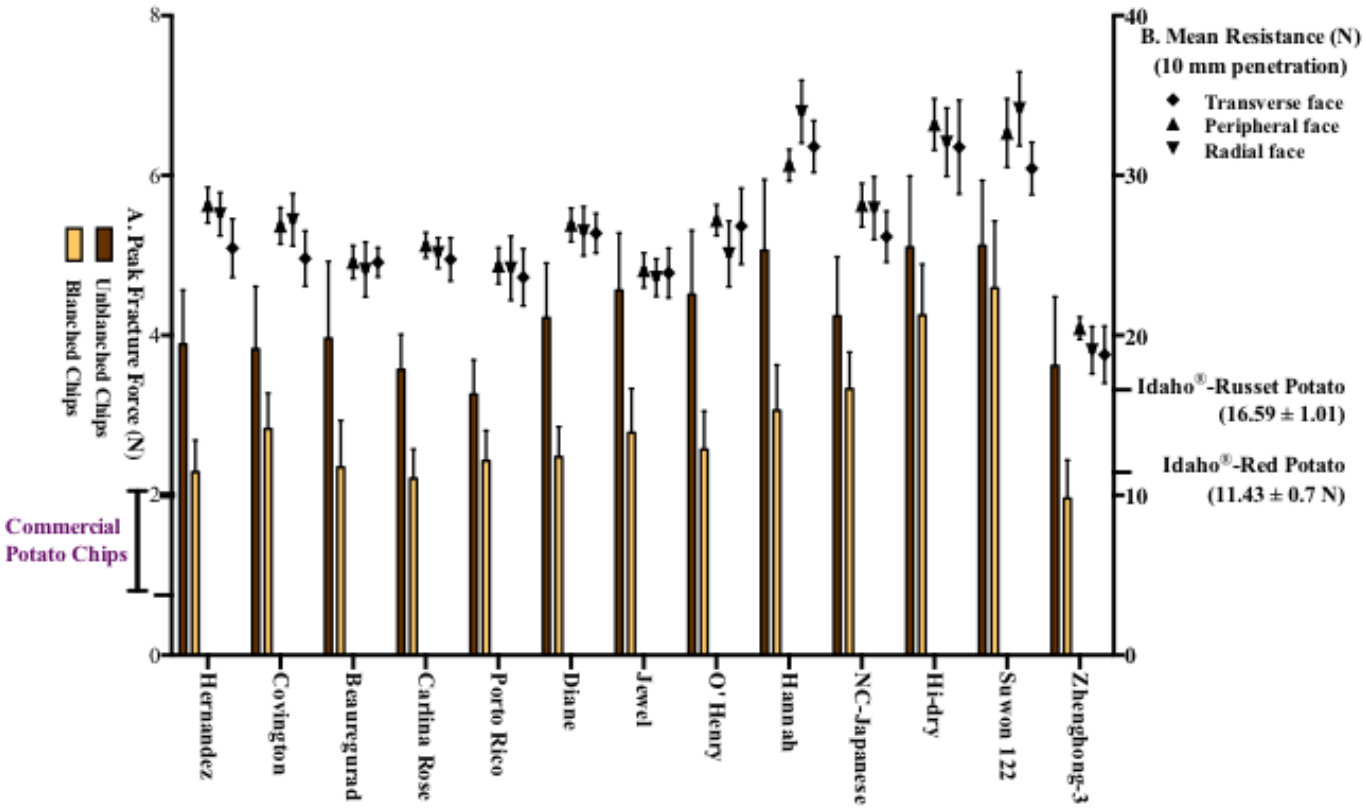

Figure 3. Comparisons of fracturability of fried chips from blanched and control sweetpotato slices of the 13 cultivars, and structure-related mechanical attributes of these sweetpotatoes. A) Fracturability of fried sweetpotato chips were evaluated via the peak fracture forces measuring hardness and brittleness of fried chips (predicting their tenderness and crunchiness), which were calculated and averaged from load-displacement curves from fracture tests of fried chips (detailed in the text). The peak fracture forces (N) of blanched (yellow bars) and unblanched chips (brown bars) from sweetpotatoes of the 13 cultivars were plotted against the left Y-axis. For comparison, the range of the peak fracture forces of potato chips from a commercial-brand is marked on the left Y-axis. B) Structure-related mechanical attributes of these selected sweetpotatoes were evaluated via the mean penetrations resistances (N) through three faces (peripheral and radial faces of halved sweetpotatoes, and transverse face of end-trimmed chunks) using an adapted puncture and penetration test. The cylinder probe was programmed to travel at a constant speed of 250 mm per min to a target penetration displacement of 10 mm from where the load cell records a 0.5 N load. The average load for each 10 mm penetration was calculated from the load versus displacement graph. At least 50 individual penetrations of about 2 to 3 mm apart were made on each face for calculating the mean penetration resistance. The mean penetrations resistances (N) through the three faces of sweetpotatoes from the 13 cultivars were plotted against the right Y-axis. The mean penetration resistances perpendicular to the tuber axis through peripheral faces of the Idaho\-Russet and Idaho\-Red potatoes are marked on the right Y-axis for comparison. The error bars represent ±1 SD.

of the same cultivar has a narrow variability (SD<5% of the mean), which makes this a sensitive test to distinguish sweetpotatoes of similar structure. The mean of more than 50 average loads taken from repeated punctures and penetrations covering a given area of a particular surface of a sweetpotato chunk is named the mean penetration resistance, which represents an average resistance against the movements of the probe (that is, shear stress) in a particular volume of sweetpotato tissues.

The mean penetration resistances are parameters related to sweetpotato structure, but not to size or developmental stage. As evidenced by the linear regression curve in Figure 4B ($r^2 = 0.3175$), there were no statistically significant differences between the mean penetration resistances (through the peripheral face) of sweetpotatoes of seven size categories (34.7 to 86 mm maximal diameter, that is, up to the size of the US No. 1) from a breeding line. Thus, variations in the mean penetration resistances of similar sized sweetpotatoes from different cultivars can be expected to truly represent tissue structural differences of sweetpotatoes of these cultivars.

The triple penetration test was then used to discern texture-relevant structural differences of various sweetpotatoes for evaluating their impact on the textural qualities of fried chips. It was initially carried out on sweetpotatoes of 12 selected cultivars (excluding Zhenghon-3). Subsequent screening of 28 additional cultivars using the test identified the Zhenghong-3

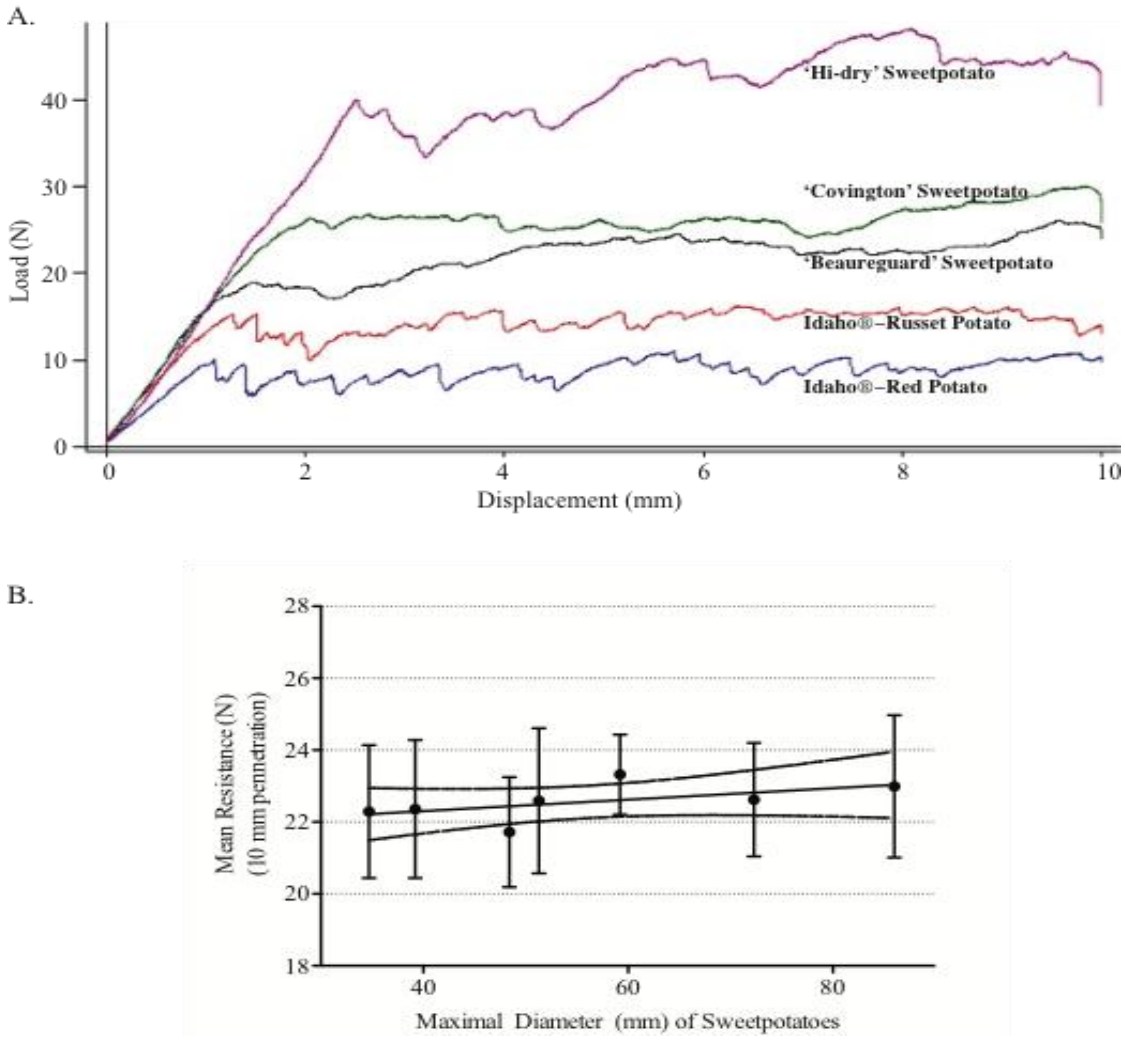

Figure 4. Demonstration of differentiation of structural differences of sweetpotatoes by the puncture and penetration test. A) Load-displacement curves of the penetration tests of sweetpotatoes of three cultivars, and potatoes of two cultivars. All five curves were recorded from penetrations perpendicular to the root or tuber long axis through peripheral face of sweetpotatoes or potato tubers. B) Linear regression curve of the mean penetration resistances (the mean of average loads of ≥50 penetrations) of sweetpotatoes of a breeding line against their sizes. The mean penetration resistances of sweetpotatoes of various sizes do not differ significantly from one another ($r^2 = 0.3175$).

sweetpotato as the one having the lowest mean penetration resistances in all three directions. The mean penetration resistances of sweetpotatoes from the 13 cultivars do not correlate with their dry matter or starch contents, which is consistent with its structure-dependent nature. Statistically significant differences among the mean penetration resistances of a sweetpotato through the three different faces were observed for some of the tested cultivars (Hernandez, Covington, O' Henry, Hannah, NC-Japanese, Suwon122, and Zhenghong-3), which may indicate a structural unevenness in these cultivars. However, this was not the case for many other tested cultivars (for example, Beauregard, Carolina Rose, Porto Rico, Diane, Jewel and Hi-dry), and may thus not be significant for breeding selection.

The mean penetration resistances through the peripheral face among the 13-sweetpotato cultivars range from 20.44 ± 0.70 N for Zhenghong-3 to 33.19 ± 0.1.61 N for Hi-dry (Figure 3). As compared with 16.59 ± 1.01 N for the Idaho®-Russet potato and 11.43 ± 0.70 N for the Idaho®-Red potato, these results indicate a harder, firmer and less brittle structure in sweetpotatoes than in potatoes.As summarized in Table 1, the mean penetration resistances of sweetpotatoes from the 13 cultivars through all three faces correlates very significantly ($P = 0.002$ or 0.003) or extremely significantly ($P<0.001$) with the peak fracture forces of their unblanched chips ($r =0.775$, $P = 0.002$, through

Table 1. Major attributes of selected sweetpotato cultivars.

Cultivars	Flesh color	Dry matter (g kg^{-1} FW)	Total starch (g kg^{-1} FW ± SD)	Starch / dry matter (%)	Amylose (g kg^{-1} starch ± SD)
Hernandez	Deep orange	193.2	82.3 ± 1.0	42.6	200.1 ± 4.2
Covington	Orange	194.4	69.4 ± 3.5	35.8	212.2 ± 4.7
Beauregard	Orange	198.2	76.7 ± 6.2	38.7	198.0 ± 3.3
Carolina Rose	Orange	202.1	102.7 ± 7.7	50.8	186.7 ± 1.3
Porto Rico	Orange mottled	206.4	156.0 ± 7.9	75.7	214.6 ± 6.0
Diane	Dark orange	209.2	93.6 ± 5.9	44.8	198.6 ± 4.1
Jewel	Deep orange	226.0	56.7 ± 3.3	25.1	181.1 ± 3.2
O' Henry	Yellow	229.0	139.6 ± 1.1	61.0	195.6 ± 1.4
Hannah	Cream	254.3	191.7 ± 1.3	75.5	181.1 ± 3.2
NC-Japanese	White	296.4	173.5 ± 4.1	58.6	207.7 ± 0.8
Zhenghong-3	Yellow cream	300.2	173.7 ± 2.9	57.9	294.3 ± 3.8
Hi-dry	Cream	306.3	273.1 ± 16.8	89.3	215.7 ± 2.6
Suwon 122	Yellow	356.0	301.8 ± 10.4	84.8	295.2 ± 5.2

peripheral face; r = 0.759, P = 0.003, through radial face; and r = 0.828, P<0.001, through transverse face). Similar results were seen for the blanched chips (r = 0.844, P<0.001, through peripheral face; r = 0.807, P<0.001, through radial face; and r = 0.771, P = 0.002, through transverse face). This suggests that sweetpotato structural attributes defining hardness, brittleness and firmness should be a primary determinant of the fracturability (also sensorial tenderness and crunchiness) of their fried chips, which is consistent with the results from the partial structural disruption test.

Impacts of sweetpotato dry matter contents, and starch contents and properties on the texture of their fried chips

Next, we investigated impacts of the dry matter content, and the total amount, composition and thermal properties of the starch in sweetpotatoes on the textural quality of fried chips. As summarized in Table 1, sweetpotatoes of various flesh colors from 13 selected cultivars have dry matter ranging from 193.2 to 356.0 g kg-1 FW, and total starch ranging from 56.7 to 301.8 g kg^{-1} FW. Sweetpotatoes from these selected cultivars provide six levels (shaded groups) of dry matter contents for evaluation. As summarized in Table 2, the dry matter and starch contents in sweetpotatoes from the 13 cultivars have a significant correlation with peak fracture forces of their unblanched fried chips (r = 0.589, P = 0.034 and r = 0.580, P = 0.038, respectively), and a very significant correlation with those of their blanched fried chips (r = 0.736, P = 0.004; r = 0.782, P = 0.002, respectively). This suggests that higher dry matter and starch contents in sweetpotatoes generally have negative impacts on textural qualities of fried chips. Moreover, the ratio of starch to dry matter contents in sweetpotatoes has a significant correlation with peak fracture forces of blanched chips (r = 0.623, P = 0.023), but not with those of unblanched fried chips (P = 0.151). In other words, a higher starch percentage in the dry matter of sweetpotatoes causes an adverse impact on the fracturability (a higher peak break force) only in fried chips from blanched slices, in which the gelatinization of starch and partial structural disruption should have helped reduction of the peak break force. Thus, impacts of starch and dry matter content on textural qualities of fried chips may be secondary to those of relevant structural attributes as the adverse impact of a higher starch to dry matter ratio appeared to be masked in fried chips from structurally intact unblanched slices.Interestingly, the amylose contents of starch showed no significant correlation with peak fracture forces of unblanched and blanched sweetpotato chips (P = 0.919 and 0.311, respectively), and should thus have little impact on chip texture.

As shown in Table 3, a large variation of gelatinization temperatures was observed among 10 tested sweetpotato starch samples. The peak (T_p) and conclusion (T_c) gelatinization temperatures of these 10 starch samples showed significant correlation with the peak fracture forces of unblanched fried chips (r = 0.660, P = 0.038; r = 0.670, P = 0.034, respectively) made from sweetpotatoes of these 10 cultivars. It is likely that a lower T_p and T_c of sweetpotato starch allowed a greater degree of gelatinization of the starch in their derived chips under the frying conditions, and thus less hardness and greater brittleness of the chips. The gelatinization onset (T_o) and T_c of these 10 starch samples also showed significant correlation with the peak fracture forces of their blanched fried chips (r = 0.668, P = 0.035; r = 0.682, P = 0.030, respectively) made from these

Table 2. Summary of correlation coefficients between major sweetpotato attributes and peak fracture forces of fried chips.

Parameter	With peak fracture forces of unblanched chips		With peak fracture forces of blanched chips	
	Pearson r	P	Pearson r	P
Dry matter content	0.589	0.034	0.736	0.004
Total starch content	0.580	0.038	0.782	0.002
Starch / dry matter	0.422	0.151	0.623	0.023
Amylose content	0.031	0.919	0.305	0.311
Starch gelatinization temperature				
T_o	0.591	0.072	0.668	0.035
T_c	0.670	0.034	0.682	0.030
T_p	0.660	0.038	0.586	0.075
Mean penetration resistances through				
Peripheral face	0.775	0.002	0.844	<0.001
Radial face	0.759	0.003	0.807	<0.001
Transverse face	0.828	<0.001	0.771	0.002

Table 3. Thermal analysis of gelatinization and retrogradation of starch from selected sweetpotato cultivars by differential scanning calorimetry (DSC).

Cultivars	Gelatinization				Retrogradation			
	T_o (°C)	T_p (°C)	T_c (°C)	Δ H (J/g)	T_o (°C)	T_p (°C)	T_c (°C)	Δ H (J/g)
Hernandez	55.3	64.5	61.7	2.9	52.7	66.5	59.6	0.8
Covington	60.6	81.5	66.3	10.0	51.2	63.9	59.9	0.9
Beauregard	64.4	81.3	71.5	13.3	51.8	65.2	59.6	2.9
Porto Rico	57.8	70.0	62.4	7.1	50.8	66.5	58.7	2.1
Diane	64.6	85.8	69.8	15.2	54.0	70.7	60.1	3.9
O' Henry	70.2	86.8	75.7	14.0	52.1	67.2	60.0	3.5
Hannah	60.3	80.3	68.5	12.3	52.3	67.4	60.2	1.7
NC Japanese	72.9	84.8	77.9	9.4	51.3	69.0	59.7	4.8
Hi-dry	69.7	89.5	74.2	12.6	52.2	66.6	59.7	2.5
Suwon 122	73.0	87.1	80.1	12.2	51.6	67.6	60.0	3.8

T_o, T_p and T_c: onset, peak, and conclusion gelatinization or retrogradation temperature (°C); ΔH: enthalpy. Values are average of at least four measurements of starch samples from two crops grown at different locations during two seasons.

cultivars. A lower T_o and T_c may have favored a higher degree of gelatinization of the starch in blanched slices under the same blanching conditions, and thus less hardness and greater brittleness of their derived chips. The gelatinization enthalpy of starch from selected sweetpotato cultivars showed large differences (2.9 J/g for Hernandez starch to 15.2 J/g for Diane starch), but no significant correlation with peak fracture forces of either unblanched or blanched chips. The thermal retrogradation properties among these starch samples, except the enthalpy, vary only within a narrow range, and have no significant correlation with the chip brittleness and hardness texture.

DISCUSSION

This study evaluated several major attributes of sweetpotatoes from 13 selected cultivars on textural qualities of their fried chips. The results indicates that sweetpotatoes having dry matter contents from ~ 19 to 22.6% (F.W.), total starch lower than ~10% (F.W.), low starch gelatinization temperatures similar to those in Covington and Hernandez sweetpotatoes, and tissue structural properties such as seen in the Zhenghong-3 sweetpotato could have the best potential for improved textural qualities of fried chips. The dry matter content in sweetpotatoes has a major impact on textural qualities of

fried chips, but is not the sole determinant as in potato. Potatoes having dry matter contents of 21.7 to 25.1% with 22.7% as the optimum are best suited for making quality chips (Lisin´ska, 1989). In contrast, a dry matter content of 22.6% in sweetpotatoes seems to be the upper limit for making chips of appropriate fracturability. All the eight sweetpotatoes cultivars (Hernandez, Covington, Beauregard, Carolina Rose, Porto Rico, Diane, Jewel and O'Henry) having starch (~57 to156 g kg^{-1}) and dry matter (~193 to 229 g kg^{-1}) contents lower than or within the range of the optimal values (~150 to 190 g kg^{-1}, and 217 to 251 g kg^{-1}, respectively) for potatoes best suited for making quality chips (Simmonds, 1977) also yielded chips with more favorable fracturability. On the other hand, sweetpotatoes having too low a dry matter content and high moisture may not be desirable either, as this could lead to higher retention of oil in fried chips (Hoover and Miller, 1973; Padmaja, 2009).

Compared to the compositional attributes, structure-related attributes of sweetpotatoes seem to have a much greater impact on textural qualities of fried chips. This is evidenced by the effective improvement of chip fracturability through partial structural disruption before frying, and by a very significant correlation between increased structural penetrability in sweetpotatoes and better fracturability of their fried chips. Blanching (especially in lightly acidic water), surface-drying, and freezing and thawing have long being recognized to be a very effective way to reduce hardness and to improve the textural quality of fried sweetpotato chips (Padmaja (2009). These mostly physical treatments were shown in this study to partially disrupt tissue structures of sweetpotato slices, and to effectively improve fracturability (hence textural qualities) of fried chips. It is estimated that unfavorable structure-related attributes of sweetpotatoes might be responsible for ~25 to 40% reduction in fracturability (predicting similar extent of sensorial tenderness and crunchiness) of chips made from 11 out of 13 tested cultivars.

The relevance and importance of tissue structures to culinary texture have been well known for many fruits and vegetables, including potato tubers (Reeve, 1970). Although, the anatomical nature of sweetpotatoes as swollen roots has been long recognized, very few attempts have been made to understand impacts of their unique structural attributes on culinary textures. Some unique structural attributes of sweetpotatoes, such as thickness of the cambium ring and degree of its lignification, and the amount and dispersion patterns of xylem strands, can be expected to have significant impacts on their culinary textures. After all, sweetpotato storage roots are primarily consumed or used as a structured food item, rather than their individual chemical components.

It is, however, very difficult to directly quantify microscopic attributes of sweetpotato structures for statistical analyses of their impacts on culinary textures, or for breeding selection. Since culinary textures are sensorial experience derived from physical interactions or behaviors of cooked food items in the mouth, the mechanical expression of the sweetpotato tissue structure as a whole becomes functionally more relevant to culinary textures. The adapted puncture and penetration test quantify such structural mechanical attributes of sweetpotatoes that can be predicative of their culinary performance, and may thus be used as a rapid method to screen clones than may have desirable culinary performance. This study also showed that sweetpotato storage roots generally have a much less penetrable structure than potato tubers, which is consistent with the report that the hardness of sweetpotatoes is about two and half times more than that of potato (Yee et al., 2012). The relatively more penetrable structure in the Zhenghong-3 sweetpotato still had a penetration resistance (perpendicular to the root axis via peripheral face) of about 23% (20.44 ± 0.70 versus 16.59 ± 1.01 N) more than that of the Idaho®-Russet potato.

The flavor quality of sweetpotatoes has been argued to be the key to sweetpotato consumption, and has been included in quality evaluations in breeding programs (Kays, 2006; Kays and Wang, 2002). However, flavor as chemical interactions of food in the mouth is only one part of the mouth-feel of a food item. Many food quality attributes from physical interactions in the mouth such as fracturability and roughness are clearly related to tissue structures of uncooked food items (Reeve, 1970). Therefore, evaluation of tissue structures, especially using simple mechanical measurements, should be also included in quality evaluation of sweetpotatoes in breeding programs, especially those for breeding specialty cultivars best suited for certain food uses.

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Conflict of Interests

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

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Full Length Research Paper

Cation availability and electrochemical conditions in oxisols modified by land use and management systems in the region of Triângulo Mineiro, Brazil

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Management and land use is one of the practices that tend to modify the chemical characteristics of the topsoil by tillage and deployment of diverse cultures isolated or consortium. From this perspective, the objective of this study was to evaluate changes in the availability of cations (calcium, aluminum and magnesium) and influence the potential of zero charge and the point and salt effect null in oxisols under different management systems and use (preserved Cerrado, eucalyptus monoculture, corn under no-tillage and pasture with baquiária grass) in the Triângulo Mineiro region. Noting that the use and soil management changes the electrochemical conditions and a correlation between the availability of calcium and magnesium, with potential of zero charge and the point and salt effect null of the soils, but shows no correlation with the organic matter for the Cerrado soils in use with corn, pasture, eucalyptus and native Cerrado vegetation.

Key words: Nutrients, potential of zero charge, point of zero salt effect, Cerrado.

INTRODUCTION

The Cerrado is located primarily in central and west-central Brazil and covers 1.8 million squared kilometer. Approximately 85% of Cerrado soils are oxisols (Demattê and Demattê, 1993), consisting primarily of clay (1:1) and oxides (typically iron and aluminum in the clay fraction). Hydroxyl (OH^-) groups on the surface of the clay and on the broken edges of kaolinite particles mean that charges are largely pH dependent and can be considered as variables (Chaves, 1999). Organic matter and amorphous materials in the soil also affect charge availability (Van Raij, 1973).

Point zero charge (PZC) occurs when the balance between positive and negative charges is zero and can determine the double-layer potential and charge distribution of the soil (Van Raij, 1973; Chaves, 1999). At PZC, soil aggregation and disaggregation are based on

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Table 1. Physical and chemical characteristics of the Oxisol under different managements and uses with cultivation of pasture, corn, eucalyptus and savanna in two layers 0.0 - 0.2 and 0.2 - 0.4 m soil in the region of Minas Gerais, Brazil.

Parameter	Sand	Silt	Clay	pH	P	Mg^{2+}	Ca^{2+}	K^{1+}	Al^{+3}	H+Al
		g kg^{-1}			mgm^{-3}			$cmol_c$ dm^{-3}		
0.0 -0.2 m										
Pasture	780.25	54.75	164.75	6.1	37.5	0.2	1.5	0.07	0	1.6
Corn	674.25	29.75	296.00	5.5	82.4	0.4	2	0.09	0	2.4
Eucalyptus	794.75	44.00	161.25	5.2	4.5	0.3	0.3	0.09	0.4	3.3
Savanna	783.50	25.25	190.75	5.1	1.5	0.1	0.1	0.06	0.4	2.7
0.2 - 0.4 m										
Pasture	785.75	41	173.7	6	4.2	0.1	0.8	0.07	0	1.6
Corn	698.25	32	270	5.5	17.4	0.3	1.1	0.05	0	2.4
Eucalyptus	794.75	44	161.25	5.2	4.5	0.3	0.3	0.09	0.4	3.3
Savanna	759.25	25.75	215.5	5.5	0.7	0.1	0.1	0.05	0.3	2.3

pH water (Soil Acidity); Al^{3+}, Aluminum; K^{+1}, Potassium; Ca^{+2}, calcium; Mg^{+2}, magnesium; P, Phosphorus - P_2O_5; H+Al, pH in SMP.

electrochemical changes (Padro, 2003). Another important parameter affecting the electrochemical behavior of the soil is the point of zero salt effect (PZSE) (Mendonça, 1998). PZSE is defined as the pH at the intersection of two or more potentiometric titration curves from solutions with different ionic strengths (Alleoni and Camargo, 1992).

PZSE and PCZ values in Cerrado soils are usually similar and are therefore good electrochemical parameters (Benites and Mendonça, 1998). Despite this similarity, it is still necessary to distinguish between the pH of PCZ and PZSE (Fernandes et al., 2008), correlations with soil properties (Silva et al., 1996) and management systems that contribute to increases in organic matter, availability of exchangeable Al and nutrients (Teixeira et al., 1994).

Thus, the objective of this study was to evaluate available Ca^{2+}, Al^{3+} and Mg^{2+} and soil organic carbon (C-org) and correlate these with pH of PCZ and PZSE in oxisols under different land use and soil management systems in the Triângulo Mineiro region of Brazil.

MATERIALS AND METHODS

Area characterization

The study area was conducted in the farm Santa Teresinha, located in the region of Triângulo Mineiro, Minas Gerais, Brazil (19° 12'11 "S and 48° 11'30" W) in the Ribeirão Bom Jardim basin on the left tributary of the Uberabinha River. The average elevation of the region is 830 m and the climate is tropical rainy with dry winters (Aw) (Antunes, 1986). The soil was classified as yellow oxisol, according to Embrapa (2000).

The experiment was completely randomized (CRD - 4x2 factorial) with four land use types (Cerrado - CE, Pasture - PA, Corn - CO and Eucalyptus - EU), two soil layers (0.0 – 0.2 and 0.2 – 0.4 m) and four replications. The environments studied had distinct land use and management types: (a) native Cerrado with woody vegetation and dark soil, due to accumulated organic material (19º12'51. 54"S latitude and 48º08'04.17"W longitude); (b) Eucalyptus: planted 30 years before the study (in place of native Cerrado) with dark soil due to accumulated vegetation and leaf litter (19º12´40.01"S latitude and 48º08´34.90"W longitude); (c) Corn (19º12'40.01" S latitude and 48º08'34.90"W longitude) and (d) Brachiaria pasture (19º13'00.22"S latitude and 48º08'24.80" W longitude). The corn and pasture areas employed a no-till system and a crop rotation of 1 year of corn followed by 5 years of pasture, Santa Fe system (Embrapa, 2000). The pasture was fertilized annually with turkey litter. Soil samples were collected four times at different points within a 1-hectare area and at depths of 0 - 0.2 and 0.2 - 0.4 m. These samples were homogenized into a composite sample which was tagged and sent to a laboratory for physical and chemical testing according to the methodology recommended by Embrapa (1997) (Table 1).

Variables and statistical analysis

Al^{3+}, Mg^{2+} and Ca^{2+} availability in soil solution and C-org were evaluated using methodologies recommended by Embrapa (1997). PZSE was determined by first establishing potentiometric titration curves at different concentrations of NaCl (0.1, 0.01, 0.001N) and of NaOH (0.8, 1.6, 2.4, 3.2, 4.0 meq H^+) in solution. PZSE was then found by determining the intersection of the titration curves where adsorption of H^+ and OH^- is equal (RAIJ, 1973). All PZSE results curves fit the model with R^2 greater than 0.90.

The estimated Point of Zero Charge PZC(est) was determined using the equation PZC(est) = pH KCl - pH H_2O (Keng and Uehara, 1974). Here, ΔpH represents the difference between the pH of soil in 1M KCl and the pH of soil in H_2O, both at a 1:2.5 ratio of soil:solution (Tan, 1982).

The results were submitted to homogeneity of variance and normality of residuals tests and then analyzed by F-test. Significant means were then compared by the Tukey test ($p < 0.05$).

RESULTS AND DISCUSSION

Significant differences (p <0.05) in Ca^{2+} availability were found between different soil layers and environments (Figure 1).

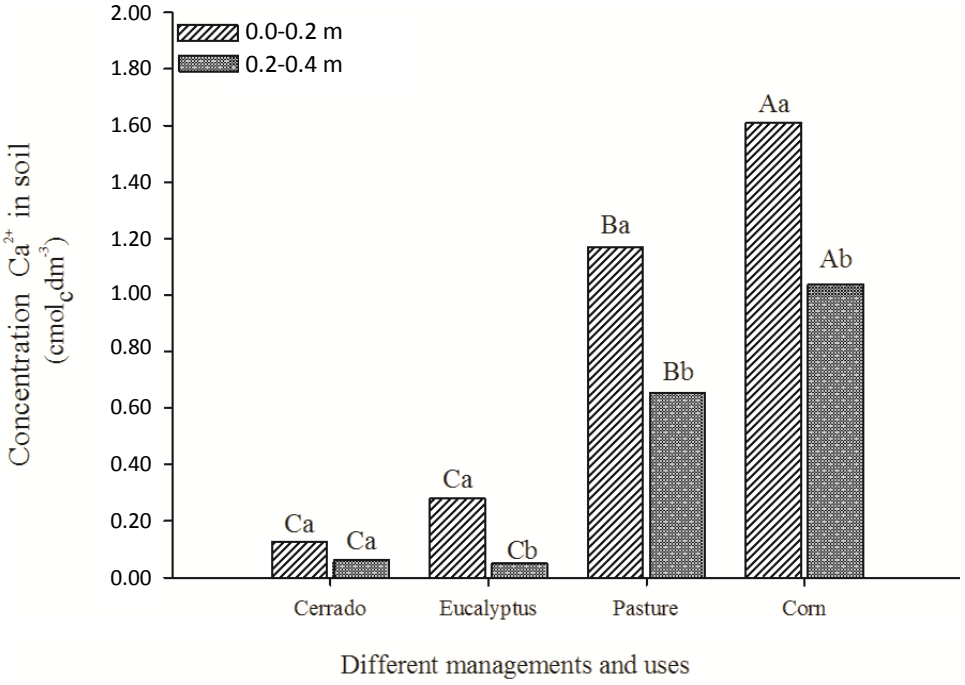

Figure 1. Availability of Calcium (Ca^{2+}) ($cmol_c$ dcm^{-3}) in oxisoils of different managements and land uses (Savanna, Eucalyptus, Pasture and Corn) in two depths (0-0.2 m and 0.2-0.4 m). The uppercase letters represent the different uses and management of land, while the lowercase letters represent the two depths; when they are distinct, distinguish among themselves by Tukey test ($p > 0.05$).

Ca^{+2} concentrations in the top 0.2 m of the soil was 43.39% higher than in the 0.2 - 0.4 m. Available Ca^{+2} were higher in CO and PA than in CE and EU because of the fertilizer amendments and crop rotations employed in these land use types.

Even though soil Ca^{+2} availability was lower in CE and EU, it was still sufficient for the requirements of the vegetation. The absence of animal and machine traffic in these environments meant that organic matter was conserved and could provide nutrients to the soil.

Significant differences in Mg^{2+} and Al^{3+} levels were also observed between soil layers and environments, but no interactions were observed between them (Table 2). Mg^{2+} was lowest in CE and significantly different from the other environments. Specifically, concentration of this macronutrient in CE was 71.15% less than in CO, which had the highest Mg^{2+} concentration.

This result is due to the absence of soil amendments in CE, which would increase Ca^{2+} and Mg^{2} levels (Fageria, 2001). Low levels of Ca^{2+} and Mg^{2+} in CE and EU led to higher levels of available Al^{3+} with greatest concentrations in the 0.0 - 0.2 m layer. These results corroborate those from a study by Souza and Alves (2003) on available Al^{3+} in Cerrado soils.

Therefore, there is a definite relationship between increasing and decreasing Ca^{2+}, Mg^{2+} and Al^{3+} in the soil. The relationship between Ca^{2+} and Mg^{2+} is positive, demonstrating that these elements increased jointly in the evaluated soils (r = 0.708). The relationship between Ca^{2+} and Al^{3+} was negative given that increasing Ca^{2+} caused available Al^{3+} to decrease (r= -0.659).

The pH values of PZC (est) and PZSE responded the same whether in water or KCl (Figure 2). The PZC (est) values for PA and CO were 23.63 and 24.27% higher than in CE while PZSE values for PA and CO were 55.83 and 54.30% higher than in CE.

According to Meurer (2010) and Van Raij (1973), lower PZC (est.) and PZSE values in CE are a result of higher C-org concentrations. This occurs because C-org can bind clay minerals, reducing positive charges and increasing negative ones.

Nevertheless, C-org was lowest in PA, followed by CEand EU and significantly higher in CO (Figure 3).

Additionally, there was no correlation between available C-org and pH - KCl (r = 0.154), pH - H2O (r = 0.149) and PZC (r = 0.143).

One possible explanation is that tropical soils have a higher ratio since iron and aluminum oxides influence soil PZSE and PZC. According to Silva et al. (1996), the absence of this interaction is due more to the type and degree of SOM (C-org) decomposition and interaction with the soil than C-org quantity.

The interaction between PZC (est.) and PZSE was positive (r= 0.54), which corroborates the results of a

Table 2. Availability of aluminum (Al^{3+}) and magnesium (Mg^{2+}) in oxisol with the uses of Savanna – CE, eucalyptus – EU, pasture- PA and corn – MI at 0-0.2 m and 0.2-0.4 m in soil profile, Fazenda Santa Terezinha, Triângulo Mineiro region.

Soil uses	Mg^{2+}			Al^{3+}		
	0 - 0.2	0.2 - 0.4	Average	0 - 0.2	0.2 - 0.4	Average
Savanna	0.11	0.08	0.091^{B}	0.45	0.28	0.362^{B}
Eucalyptus	0.31	0.14	0.223^{A}	0.40	0.30	0.350^{B}
Corn	0.40	0.23	0.248^{A}	0.14	0.05	0.000^{A}
Pasture	0.30	0.20	0.312^{A}	0.00	0.00	0.093^{A}
Média	0.279^{A}	0.158^{B}		0.246^{B}	0.156^{A}	

*Availability of Al^{3+} and Mg^{2+} expressed in $cmolcdm^3$ and the layers meter (m). Averages followed by distinct capital letters in the column identifying management systems and land use. Identifying the row and lower layers of the soil profile, differ by Tukey test ($p > 0.05$).

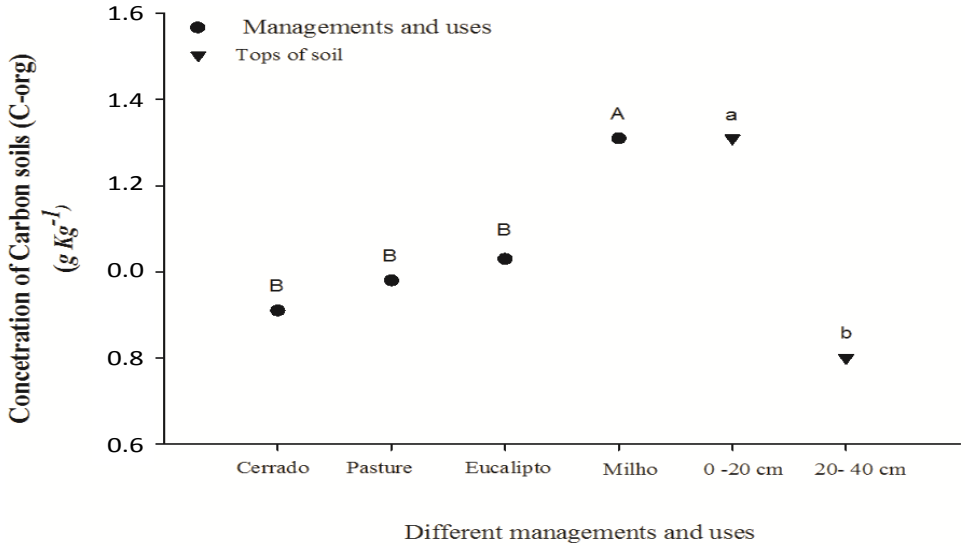

Figure 2. Values of potential zero charge (PZC est), pH in water and in KCl and the point of null saline effect (PESN) of the oxisoils in different managements and land uses (Savanna, Eucalyptus, Pasture and Corn). The uppercase letters represent the uses and management of land, when they are distinct, distinguish among themselves by Tukey test ($p > 0.05$).

study by Fernandes et al. (2008) on different soils of the Caatinga (dry savanna unique to northeastern Brazil). According to Benites and Mendonça (1998), PZC (est.) and PZSE are similar and positively correlated in highly weathered soils where electric charges are almost entirely pH dependent.

In addition, available Ca^{2+} (r = 0.82) and Mg^{2+} (r = 0.49) were significantly correlated with PZC (est.) and Ca^{2+} (r= 0.41) was significantly correlated with PZSE. This demonstrates that the electric potential of the soil was more significantly associated with the availability of these cations.

This positive correlation between PZC and cations is due to electrochemical phenomena of soils with variable charges, which affect properties such as cation exchange and nutrient availability (Fontes et al., 2001). Greater availability of Ca^{2+} and Mg^{2+} increases the specific adsorption of the inner sphere complex, leading to the formation of positive charges on colloid surfaces (Sposito, 1989). Moreover, the cation exchange capacity (CEC) of oxisols is pH dependent, affecting negative charges arising from the reaction of lime.

There were no significant differences in PZC (est.) and PZSE relative to the two soil layers studied. Similar results were found in a study (Demattê, 1993) in Planaltina, Brazil that compared an area of native Cerrado vegetation to 16 areas with different types of soil management and land use in clayey Oxisol. However, Van Raij (1973), working with the Ap and B2 horizons of an Oxisol, found differences in PZC (est.) in the surface

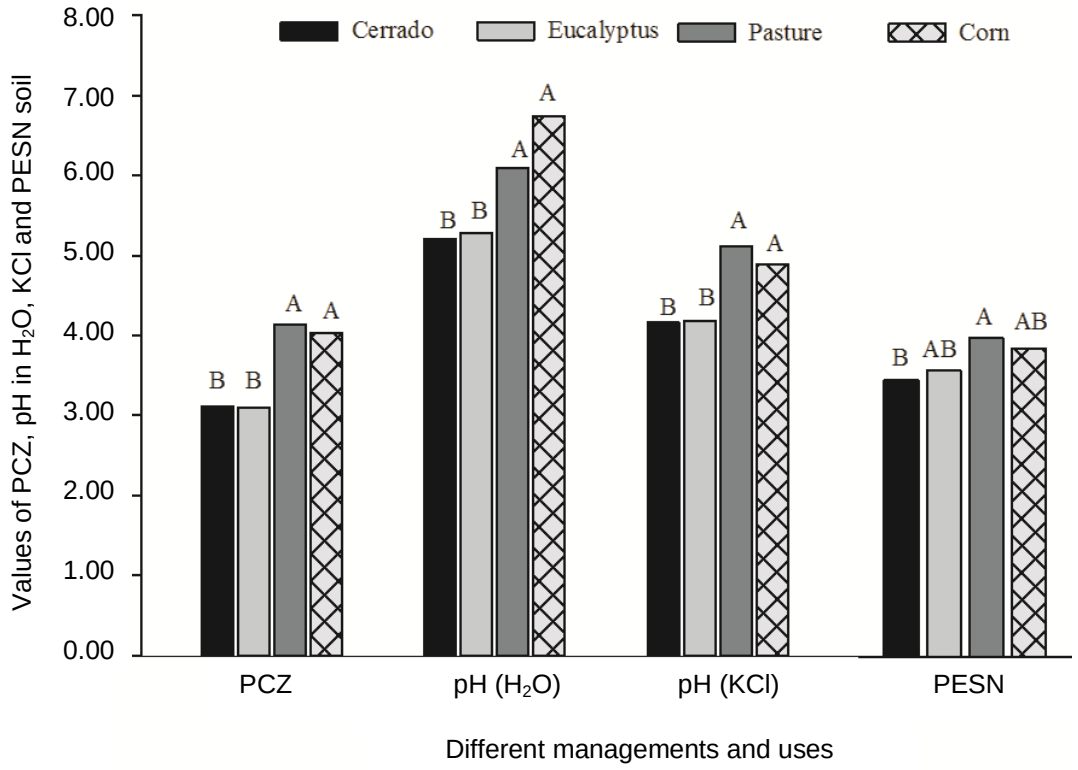

Figure 3. Effect of the use and management of soil in two soil depths (0-0.2 and 0.2-0.4 m) in the levels of soil organic carbon (C-org - g kg^{-1}). Points on the graph marked with different uppercase letters differ from each other between the types of use and soil management. Points marked with lowercase letters distinguish among each other soil depths by the Scoott-Knott test (p <0.05).

Table 3. Delta pH (ΔpH) in Oxisol with usage with savanna, eucalyptus, pasture and corn in layers of 0-0.2 0.2-0.4 m in the Santa Terezinha, Triângulo Mineiro region.

Management and land use [1]	ΔpH*		Average
	0 - 0.2 m	0.2 - 0.4 m	
Savanna	-1.052	-1.022	-1.042^{A}
Eucalyptus	-1.152	-1.040	-1.096^{AB}
Pasture	-0.927	-1.027	-0.982^{AB}
Corn	-0.900	-0.810	-0.850^{B}

* Delta pH (ΔpH): difference between the pHKCl and pH H_2O [1] - Table averages accompanied with uppercase column identifies the land uses and managements when distinct differ among themselves by Tukey test (p > 0:05).

layers due to organic matter accumulation on the soil surface.

ΔpH was negative (Table 3) in all environments. This indicates the predominance of negative charges on the surfaces of minerals and organic matter, which increase CEC. This situation occurs in tropical soils due to acidity and significant quantities of oxides, mainly iron and aluminum (Meurer, 2010). The PCZ (est.) of these oxides, both in crystalline (gibbsite, hematite and goethite) and amorphous forms ranges from 5.5 to 6.0 regardless of cation content (Demattê, 1993).

Conclusion

Land use and soil management tend to alter the electrochemical conditions of the soil with a positive correlation between Ca^{+2} and Mg^{+2} availability and PZC (est.) and PZSE. However, PZC (est) and PZSE are not correlated with C-org in Oxisol soils under CO, PA, EU

and CE land use in the Triangulo Mineiro region of Brazil.

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Conflict of Interests

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

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Full Length Research Paper

Antibiosis resistance of soybean genotypes to *Diabrotica speciosa* (Germar, 1824) (Coleoptera: Chrysomelidae)

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This research aimed to discriminate antibiosis-type resistant soybean genotypes to *Diabrotica speciosa* (Germar, 1824). Six soybean genotypes were used, according to previous selection, which were: IAC 100, BRSGO 8360, IGRA RA 626 RR, DM 339, PI 227687 and PI 274454. A completely randomized design was adopted, with six treatments and 10 replicates. Each replicate was constituted by 10 soybean plants and 10 *D. speciosa* larvae, in the initial infestation, doubling the number of plants in the transferring, 10 days after the initial infestation, using fine-grained vermiculite as substrate. In this experiment, the following characters from *D. speciosa* were assessed: Number of males, females and total of emerged insects, total survival (percentage), sex ratio, male and female weight (mg), larva to adult period and longevity (without food). The genotypes DM 339, PI 274454, and PI 227687 show antibiosis resistance against *D. speciosa*.

Key words: *Glycine max*, host plant resistance to insects, insects' biology, corn rootworm, pest management, soil pests.

INTRODUCTION

The *Diabrotica* genus (Chevrolat, 1844) (Coleoptera: Chrysomelidae) includes some of the most harmful pests that occur in the whole America (Cabrera Walsh, 2003). In the last decades, the relevance of the species from this genus has increased in consequence of serious injuries caused by native species on plants from different crops in the American continent (Krysan, 1986; Tollefson, 1998). *Diabrotica speciosa* (Germar, 1824) is already established in many Brazilian states, and has economic expression as it is a polyphagous pest by causing losses to crops such as soybeans (*Glycine max* [L.] Merril), beans (*Phaseolus vulgaris* L.), maize (*Zea mays* L.), peanuts (*Arachis hypogaea* L.) and potatoes (*Solanum tuberosum* L.), between others, due to the injuries caused on the plant. This species is also a vector of plant pathogens, primarily viruses (Laumann et al., 2003).

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D. speciosa has a holometabolous life cycle (Milanez and Parra, 2000). Females lay eggs in the soil, around the plants. The incubation period ranges from six to eight days. The larval stage goes through three instars, and its mean duration period is 18 days. After completing the larval stage, the insects are directed to the soil, where they build a pupal chamber to develop into pre-pupae and pupae. The pre-pupae mean duration period is five days, and the pupal stage is seven days. The life cycle varies from 24 to 40 days (Ávila and Parra, 2001; Milanez and Parra, 2000).

The larvae of this chrysomelid feed and develop on the roots and tubers of several crops (Milanez and Parra, 2000). The feeding damage can cause developmental delay and increase in the susceptibility of the plants to lodging (Marques et al., 1999). Conversely, the adults feed on aerial parts of plants, such as leaves, stems, fruits, and pollen of crops and wild plants (Gassen, 1989).

This insect species is one of the most abundant and harmful pests in Brazilian crops (Christensen, 1942; EPPO, 2005) and presents a hazard to the European continent, as well other species from North America (EPPO, 2005).

To control this pest, the main additional problem is the environmental risk associated to the chemical control by synthetic insecticides. Excessive insecticide applications lead to resistance in pests, human health risks and hazards for natural enemies and pollinators (Ávila and Nakano, 2000). This modality demands relatively higher amounts of active ingredient per area, and hence increases the costs and it may contaminate the groundwater, mainly in arenaceous soils (Pereira et al., 2005).

In modern agriculture, host plant resistance is an integral component, if not the basis, of arthropod pest regulation in integrated pest management (IPM) programs (Panda and Kush, 1995; Smith, 2005; Stout, 2007). The plant resistance category known by antibiosis occurs when the negative effects from a resistant plant affect the biology of an arthropod, when it uses the plant to feed upon. The antibiotic effects from a resistant plant vary from moderate to lethal, and may result due to chemical and morphological mechanisms (Smith, 2005).

Introductions of the soybeans lines PI 171451, PI 227658 and PI 229358 have been used since the beginning of the 1970's decade as resistant sources to insects which feed on leaves, as *D. speciosa* (Rezende and Miranda, 1980).

Thus, this research aimed to discriminate antibiosis-type resistant soybean genotypes to *D. speciosa*.

MATERIALS AND METHODS

The experiment was conducted at the Laboratório de Resistência de Plantas a Insetos, from the Departamento de Fitossanidade of the Faculdade de Ciências Agrárias e Veterinárias – FCAV/UNESP, under temperature conditions of 25 ± 2°C, relative humidity of 70 ± 10% and a photophase of 12 h.

Insects breeding

The *D. speciosa* rearing method used for the elaboration of this experiment was carried out according to the methodology proposed by Ávila et al. (2000). The adult insects were collected in a bean crop.

To obtain *D. speciosa* eggs, Petri dishes (14 cm in diameter × 2 cm in height) were used, containing in their interior moistened cotton and, on this, black gauze as substrate for oviposition. The eggs were removed from the oviposition substrate by rinsing the gauze in running water into synthetic polyester fabric of 900 cm² (mesh size of 0.03 × 0.03 mm), where the eggs were retained. In order to avoid the contamination by fungi and other microorganisms during the incubation period, the eggs were treated with copper sulfate solution ($CuSO_4$) at 1%, per two minutes, and later, they were transferred into Petri dishes (9 cm in diameter and 1 cm in height), lined with moistened filter paper (Ávila et al., 2000).

For the rearing, an initial infestation of neonate larvae was performed in plastic containers (17 cm in diameter × 9 cm in height). First, the containers were filled with 40 g of fine-grained vermiculite moistened with 50 g of deionized water. Afterwards, 70 plants of AL-Piratininga variety were placed on the moistened vermiculite, and subsequently 70 larvae were put on the maize roots for feeding and developing. Ten days later, the larvae were transferred to larger plastic containers (27 cm in length × 16 cm in width × 9.5 cm in height).The amounts of deionized water, vermiculite, and plants were doubled, aiming to provide healthy plants and larger space for the developing of larvae and pupae of *D. speciosa*, until the adults emergence. The maize seeds were treated with the fungicide carbendazim + thiram (Derosal Plus®), applying 200 ml of the commercial product to 100 kg of seeds.

The *D. speciosa* adults were fed on bean leaves of IAC Carioca-Tybatã variety, which were placed in glass cages (40 cm long × 30 cm high × 30 cm wide)

Bioassay

For the experiment conduction, the sequence and rearing methodology formerly reported were followed, using, although, plants from the studied soybean genotypes, as follows: IAC 100, BRSGO 8360 (susceptible), IGRA RA 626 RR (resistant), DM 339, PI 227687 (resistant) and PI 274454 (resistant), which were screened from the results obtained in a previous feeding preference test for adults (Costa et al., 2012). The soybean seeds were treated with the fungicide carbendazim + thiram (Derosal Plus®), by applying 200 ml of the commercial product to 100 kg of seeds.

A completely randomized design was used, with six treatments (genotypes) and 10 replications. Each replication was constituted by 10 soybean plants and 10 *D. speciosa* larvae, in the initial infestation, totaling 100 plants and 100 larvae per treatment. On the transferring occasion (10 days after the initial infestation), the surviving larvae were relocated to another container, with similar quantities of vermiculite and water, as previously mentioned, but only the number of plants were doubled, in order to provide greater food amount (soybean roots) for the insects in the immature phase. For both process, initial infestation and transferring, plastic containers of 12 cm in diameter × 9.5 cm in height were used.

The following characters of *D. speciosa* were evaluated in the experiment: Number of males, females and total of emerged insects, survival (percentage), sex ratio, weight (mg) of males and females, using a precision analytical balance (model AS200S, Florham Park, NJ), larva to adult period, and longevity (without food).

Statistical analysis

Data obtained in these experiments were submitted to normality

Table 1. Number (mean ± standard error) of males, females and total of emerged insects of *Diabrotica speciosa* per container fed on different soybean genotypes.

Genotype	Males number	Females number	Total number of insects
IAC 100	0.50 ± 0.17^{bc}	1.20 ± 0.44^{b}	1.70 ± 0.54^{b}
BRSGO 8360	1.10 ± 0.28^{c}	1.90 ± 0.50^{c}	3.10 ± 0.74^{b}
IGRA RA 626 RR	1.30 ± 0.52^{c}	1.00 ± 0.33^{b}	2.30 ± 0.72^{b}
DM 339	0.00 ± 0.00^{a}	0.20 ± 0.13^{ab}	0.20 ± 0.13^{a}
PI 227687	0.10 ± 0.10^{ab}	0.30 ± 0.15^{ab}	0.40 ± 0.22^{a}
PI 274454	0.30 ± 0.21^{ab}	0.00 ± 0.00^{a}	0.30 ± 0.21^{a}
H	18.66**	18.62**	22.48**
p	< 0.01	< 0.01	< 0.01

Temperature: 25 ± 1ºC; R. U: 70 ± 10%; Photofase: 12 h. Jaboticabal, SP, Brazil, 2012. Means followed by different letters in column are significantly different by Kruskal Wallis' test at 5% probability.

Table 2. Total survival (%), sex ratio and longevity (mean ± standard error) of *D. speciosa* individuals fed on different soybean genotypes.

Genotype	Total survival (%)	Sex ratio	Longevity (days)
IAC 100	17.00 ± 5.39^{b}	0.71 ± 0.11	1.90 ± 0.44^{bc}
BRSGO 8360	31.00 ± 7.37^{b}	0.63 ± 0.09	2.59 ± 0.30^{c}
IGRA RA 626 RR	23.00 ± 7.16^{b}	0.43 ± 0.11	2.10 ± 0.47^{bc}
DM 339	2.00 ± 1.33^{a}	-(^{a})	0.60 ± 0.40^{ab}
PI 227687	4.00 ± 2.21^{a}	0.75 ± 0.25	0.55 ± 0.30^{a}
PI 274454	3.00 ± 2.13^{a}	0.00 ± 0.00	0.65 ± 0.43^{ab}
H	22.48**	7.89^{ns}	17.05**
p	< 0.01	> 0.05	< 0.01

Temperature: 25 ± 1ºC; R. U: 70 ± 10%; Photofase: 12 hours. Jaboticabal, SP, Brazil, 2012. Means followed by different letters in column are significantly different by Kruskal Wallis' test at 5% probability. (^{a}) Data were not analyzed due to the insufficient number of replicates.

(Shapiro-Wilk) and homocedasticity (Bartlett) tests (Silva and Azevedo, 2006). Considering that they are non-normal variables, which could not be normalized by transformations, the Kruskal-Wallis' non-parametric test (H statistic) was used. Analyses were carried out at the $P < 0.05$ level of probability.

RESULTS

Differences were observed amid the soybean genotypes in the following biological variables: Number of males, females and total of emerged insects (Table 1), total survival and longevity (Table 2), larva to adult period and females weight (Table 3). Regarding the number of emerged males ($H = 18.66$; df = 5; $P < 0.01$), the highest values were found for the genotypes BRSGO 8360, 1.10, and IGRA RA 626 RR, 1.30, whilst for the genotype DM 339 the occurrence of individuals of this sex was not recorded. Concerning the other variables cited, the genotype BRSGO 8360 highlighted with the highest values. Analyzing the number of emerged females ($H = 18.62$; df = 5; $P < 0.01$), BRSGO 8360 stood out with the highest value, 1.90, differing from all the other genotypes. No one female emerged from the genotype PI 274454. Regarding the total number of emerged insects ($H = 22.48$; df = 5; $P < 0.01$), the genotypes BRSGO 8360, IGRA RA 626 RR, and IAC 100 held the highest mean numbers, with 3.10, 2.30, and 1.70, respectively, differing from the genotypes DM 339, PI 274454, and PI 227687, which showed 0.20, 0.30, and 0.40, respectively. These data can be expressed in total survival (%) (Table 2), in which the genotypes were divided similarly into two groups. It is important to note that the total survival on BRSGO 8360 (31.00%) was 15.5 times higher than on DM 339 (2.00%).

For longevity (Table 2) ($H = 17.05$; df = 5; $P < 0.01$), the adults emerged from the genotype BRSGO 8360 stood alive for a longer time, 2.59 days, differing from those insects reared on the genotypes DM 339 (0.60 days), PI 227687 (0.55) and PI 274454 (0.65). With respect to the duration of larva to adult period ($H = 14.45$; df = 5; $P < 0.01$), BRSGO 8360, IAC 100, and PI 227687 provided the longer cycles, 31.23, 31.00, and 30.50 days, respectively, whereas the shorter cycle was verified for the genotype PI 274454, 28.67. Concerning the females weight ($H = 12.56$; df = 5; $P < 0.01$), the highest values were found for BRSGO 8360 and IGRA RA 626 RR, with

Table 3. Larva to adult period (days), and male and female weight (mg) (mean ± standard error) of *D. speciosa* fed on different soybean genotypes.

Genotype	Larva to adult period (days)	Females weight (mg)	Males weight (mg)
IAC 100	31.00 ± 0.42[b]	6.23 ± 0.81[a]	7.12 ± 0.28
BRSGO 8360	31.23 ± 0.32[b]	7.93 ± 0.46[b]	8.06 ± 0.55
IGRA RA 626 RR	29.52 ± 0.44[ab]	8.94 ± 0.48[b]	9.00 ± 0.47
PI 227687	30.50 ± 0.29[b]	5.00 ± 0.70[a]	-([a])
PI 274454	28.67 ± 0.33[a]	-([a])	8.23 ± 1.45
F	14.45**	12.56**	5.22[ns]
p	< 0.01	< 0.01	> 0.05

Temperature: 25 ± 1ºC; R. U: 70 ± 10%; Photofase: 12 h. Jaboticabal, SP, Brazil, 2012. Means followed by different letters in column are significantly different by Kruskal Wallis' test at 5% probability. ([a]) Data were not analyzed due to the insufficient number of replicates.

7.93 and 8.94 mg, respectively, which differed from IAC 100 and PI 227687, 6.23 and 5.00 mg. In Table 3, there were not data for DM 339 genotype, due to the high mortality of insects.

DISCUSSION

The results obtained in this experiment demonstrated, in general, that the genotypes DM 339, PI 227687 and PI 274454 were the least suitable for *D. speciosa* development, by expressing resistance by antixenosis to the pest, while for the genotypes IAC 100, BRSGO 8360 and IGRA RA 626 RR, favorable results for the insect development were found.

Ávila and Parra (2002), while studying the *D. speciosa* development on different genotypes, concluded that under field conditions, in the absence of a preferred host, the larvae of this species may use soybean plants as an alternative host for its feeding and breeding. In Brazil, Corseuil et al. (1974) reported that larvae of *D. speciosa* feed on soybean plants roots. Hoffmann-Campo et al. (2000) mentioned that this pest has been causing preoccupation to soybean growers from the west and southeast regions of Paraná state. According to Cabrera Walsh (2003), *D. speciosa* larvae developed well on maize, peanuts, and soybean roots.

In the 1990 decade beginning, a strain of *Diabrotica virgifera virgifera* (LeConte) became established in the USA Corn Belt, adapting itself to the maize-soybean crop rotation by the oviposition in soybean fields, which must be rotated to maize crop in the next year (Gray et al., 1998). However, the current research demonstrates the relevance of studying the host plant resistance, by detecting genotypes which highlight regarding the average, that is, express resistance. Thus, this type of experiment would be very important to the subsequent utilization of resistant genotypes in areas which the cultivation of different crops is carried out, such as in Brazil, in order to reduce the pest density in the off season. The genotype DM 339, as verified in the current research, is a resistant material, and as it is a commercial cultivar, perhaps it is an option in the attempt to decrease the *D. speciosa* population level, when, for instance, the maize-soybean crop rotation is necessary. It is relevant yet to consider that this pest species is multivoltine, and as mentioned before, it can feed on soybean roots.

Ávila and Parra (2002), when evaluating the *D. speciosa* development on different hosts, concluded that the insects' survival which fed upon soybean roots from the genotype IAC-8 was 30.1%, very close to the rate found on BRSGO 8360 in our study (31.0%). However, when comparing different hosts on the development of an insect, it is important to know the resistance of the studied genotypes, having in order the dissimilarity detected among the genotypes in this work, where the survival values ranged from 2.0 to 31.0%.

Dunbar (2011) assessed the soybean varieties effects on *D. v. virgifera*, and concluded there were no differences amidst the varieties with the genes *rag1*, *rag1/rag3* and a susceptible isoline from *rag1* variety on the survivorship, eggs production or leaf intake of the soybean varieties, and despite these varieties are resistant to *Aphis glycines* Matsumura, the same does not seem to impose natural selection against *D. v. virgifera* resistance in the maize-soybean crop rotation. The same authors affirm the varieties utilization which reduce the *D. v. virgifera* fitness, may help in the delaying of the pest insect resistance evolution. On the other hand, this research revealed genotypes expressing resistance, emphasizing the importance of the study on materials from different origins, comparing lines and cultivars, for instance.

The genotypes DM 339, PI 274454, and PI 227687 show antibiosis resistance against *D. speciosa*.

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Effect of carbohydrate source, pH and supporting media on *in vitro* rooting of banana (*musa spp*.) cv. Grand naine plantlets

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The present study was conducted in the Division of Fruit Science, SKUAST-Jammu during the year 2012 to 2013 to investigate the effect of different carbohydrate source, pH and supporting media on *in vitro* rooting of banana plantlets using MS medium with 0.1 mg/L IBA and activated charcoal. Sucrose in the medium remarkably influences the rooting of plantlets. In the absence of sucrose, culture could not survive after 3 weeks of incubation. In the sucrose containing media, 30 g/L gave the best result. Out of different pH levels tested, minimum time for root initiation with longest length of root was obtained on pH 5.5. The reduction of agar concentration from 0.8 to 0.4% in the medium improve the *in vitro* root and shoot characters as compare to other supporting structures viz., Whatman No. 1 filter paper, ordinary filter paper and brown paper.

Key words: Micropropagation, *Musa* spp, Grand Naine, *in vitro* rooting.

INTRODUCTION

Banana is the premier fruit of Asia and the pacific. It is widely grown in the tropics and sub-tropics in all types of agricultural system from small, mixed, subsistence gardens to large commercial monocultures. The crop serves in many countries as a staple food or the cornerstone of the country's economy. For commercialization, it is important that consistent supplies of good quality bananas are produced. This is achieved through clonal planting material obtained through tissue culture propagation technique. This technique provides high rates of multiplying, genetically uniform and year around availability of pest and disease-free planting material. However, before exploiting such a technique for commercial purposes, gathering information regarding the requirements for each of micropropagation and then developing an industrially practicable procedure for the best culture conditions are necessary (Anderson, 1980; Lakshmi et al., 1982). The survival of the micropropagated plants in *ex vitro* conditions largely dependant on the efficient rooting of shoots. For this reason, an optimum requirement for maximum rooting response needs to be determined for each plant.

The factors which greatly influenced *in vitro* rooting of shoot are carbohydrate sources, pH of the medium and

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supporting structures (Kitto and young, 1981). The role of pH on adventitious root formation has been associated with acidic pH (Lee et al., 1976; Stone, 1963; Williams et al., 1985), alkaline pH (Lee et al., 1976) and near-neutral pH (Mellor and Stace-Smith, 1969). Therefore, the present investigation was undertaken to evaluate the effect of carbohydrate sources, pH and supporting structures on *in vitro* rooting of banana cv. Grand Naine.

MATERIALS AND METHODS

The present study was conducted in the Division of Fruit Science, SKUAST-Jammu during the year 2012 to 2013. Sher-e-Kashmir University of Agricultural Sciences and Technology, Chatha is situated in the sub-tropical zone at latitude of 32°.4'N and 74°.58'E longitude. The altitude of the place is 332 m above sea level. Annual precipitation is about 1000 to 1200 mm. Most of the rains are received during July to October (about 70%). The mean annual maximum and minimum temperatures are 45 and10°C respectively. Summer months are hot with temperature and humidity ranging from 23.50 to 35.50°C and 53.00 to 73.50%, respectively. The winter months experience mild to severe cold conditions with an average temperature ranging from 6.50 to 21.70°C. January is the coldest month, when minimum temperature touches at 3.8°C. The highest temperature 45.0°C is recorded in the month of June. The planting material of four week old suckers of *Musa* cultivar Grand Naine was collected from the healthy true to type mother plants. For obtaining the explants material, the suckers were removed from the mother plant with a straight flat bar having a sharpened point that could be inserted between plant and the sucker. The suckers were excised and surface sterilized by using different combinations and concentration of mercuric chloride, sodium hypochlorite and ethanol individually or in combination is presented in Table 1**,** which was followed by washing of explants thoroughly in running tap water for 30 min and treatment with 10% detergent solution (Teepol, BDH) for 10 min. Suckers were cultured on agar gelled Murashige and Skoog basal medium (MS) with full strength salts supplemented with specific concentration of growth regulators [6-Benzylamino purine, Indole-3-acetic acid and Naphthalene acetic acid (NAA)] singly or in combination and 3% sucrose was used for culture establishment and shoot multiplication and MS half strength salts containing various concentration of auxins (IBA/NAA) were used for adventitious root formation. All the treatments, except those for different levels of pH, were adjusted to pH 5.8 ± 0.1 fortified with 30 g/L sucrose and 0.8% agar. Media were supplemented with 1.0 mg/L IBA and 200 mg/L activated charcoal. These were added to the media before autoclaving for 20 min. All the cultures were incubated in the culture room at 26 ± 2°C under 16 h photoperiod and about 1.5 k lux light intensity. Data recorded for different parameters viz. surface sterilization of explants, culture establishment and multiplication (Stage I & II), shoot proliferation, *in vitro* rooting (Stage III) and acclimatization to normal conditions (Stage IV) were subjected to completely randomized design (CRD). Statistical analysis based on mean values per treatment was made using analysis of variance (ANOVA) technique of CRD.

RESULTS

Effect of different carbohydrate source

The carbon energy source is inevitable in any culture medium. Sucrose is the most widely accepted carbon source. The growth of the culture is not only affected by the particular type of carbon source used, but also by its concentration (Mehta, 1980). Out of various carbohydrates tested at different levels (Table 2 and Figure 1), rooting was observed only on media containing sucrose 1 to 3%, whereas in other medium containing different carbohydrate sources cultures were dried after 3 weeks of incubation. In the sucrose containing media, cent percent rooting was observed on sucrose 3%. The maximum number of roots/shoot (6.00 cm) was obtained in sucrose 3%. The length of shoot and length of root increased with increase in concentration of sucrose whereas in case of number of leaves per shoot, the treatments did not show much variation.

Effect of pH

The pH of the medium affected substantially the *in vitro* rooting of banana shoots (Table 3 and Figure 2). Cent percent rooting was obtained in pH 5.5 and 5.8. Regarding, the number of days taken for rooting, the minimum days (6.33) were required at pH 5.5, which was closely followed by medium having pH 5.8 (6.66 days), pH 6.0 (7.66), pH 5.0 (9.33) and pH 6.5 (10.66). Likewise, maximum number of roots (7.66), length of root (8.50 cm) and length of shoot (7.10 cm) were recorded at pH 5.5, which was significantly superior to rest of the treatments. In case of number of leaves, no significant differences were obtained among different pH levels.

Effect of supporting media

The reduction of agar concentration from 0.8 to 0.4% in the medium was found to improve the *in vitro* rooting and shoot characters. The data related to the effect of supporting media on *in vitro* root and shoot growth characteristics as shown in Table 4 and Figure 3 revealed that cent percent cultures showed rooting on medium gelled with 0.4% agar which varied significantly with 0.8% agar, whereas all other supporting media such as Whatman No. filter paper, ordinary filter paper and brown paper were found unsuitable for rooting.

The time taken for root initiation was minimum (5.66 days) in medium gelled with agar 0.4% which was closely followed by treatment agar 0.8% (6.33 days) and both of them were at par. Maximum number of roots (7.00) per culture with maximum length of root (6.00 cm) was observed in the cultures rooted on medium gelled with agar 0.4%. Similar trend was obtained regarding length of shoot whereas there was insignificant effect on number of leaves obtained on shoot.

DISCUSSION

Effect of different carbohydrate source

The results obtained in the present study are in

Table 1. Standardization of surface sterilization method for banana explants.

S/No	Sterilant	Concentration (%)	Duration
1	Dipping in mercuric chloride	0.1	2 min
2	Dipping in mercuric chloride	0.1	4 min
3.	Dipping in mercuric chloride	0.1	6 min
4.	Dipping in mercuric chloride and subsequent dipping in Ethanol	0.1 70	2 min 30 s
5.	Dipping in mercuric chloride and subsequent dipping in Ethanol	0.1 70	4 min 30 s
6.	Dipping in mercuric chloride and subsequent dipping in Ethanol	0.1 70	6 min 30 s
7.	Dipping in sodium hypochlorite	5	5 min
8.	Dipping in sodium hypochlorite	5	10 min
9.	Dipping in sodium hypochlorite and subsequent dipping in ethanol and mercuric chloride	5 70 0.1	5 min + 30 s + 2 min
10.	Dipping in sodium hypochlorite and subsequent dipping in ethanol and mercuric chloride	5 70 0.1	5 min + 30 s + 4 min
11.	Dipping in sodium hypochlorite and subsequent dipping in ethanol and mercuric chloride	5 70 0.1	5 min + 30 s + 6 min
12.	Dipping in sodium hypochlorite and subsequent dipping in ethanol and mercuric chloride	5 70 0.1	10 min + 30 s + 2 min
13.	Dipping in sodium hypochlorite and subsequent dipping in ethanol and mercuric chloride	5 70 0.1	10 min + 30 s + 4 min
14.	Dipping in sodium hypochlorite and subsequent dipping in ethanol and mercuric chloride	5 70 0.1	10 min + 30 s + 6 min

agreement with the result of Amin and Jaiswal (1989a) who reported that in the absence of sucrose culture could not survive after 3 weeks of incubation and in the sucrose containing media 30 to 40 g/L gave the best result. The similar positive effect of increased level of sucrose on *in vitro* rooting has been reported for sour cherry (Snir, 1983) and walnut (Driver and Kuniyuki, 1984). Likewise, Kabir et al. (2007) reported that sucrose in 30 g/L concentration as carbon source was proved to be best regarding the growth of shoot tip explant of papaya.

Effect of pH

Though, the importance of pH in tissue culture studies was reported by Gautheret (1947), who observed pH drift

Figure 1. Effect of different carbohydrate source on in vitro rooting of banana plantlets.

Table 2. Effect of different carbohydrates on *in vitro* rooting and shoot growth of banana plantlets.

S/No	Treatment	Time taken for root initiation (days)	Culture rooted (%)	No. of roots per shoot	Length of longest root (cm)	Length of shoot (cm)	No. of leaves per shoot
1.	Sucrose 1%	13.00	75.66 (60.41)	4.00	5.66	4.90	5.66
2.	Sucrose 2%	12.00	83.33 (65.87)	5.66	6.00	5.70	6.33
3.	Sucrose 3%	11.00	100.00 (90.00)	6.00	7.00	6.20	7.00
4.	Commercial sugar 1%	-	-	-	-	-	-
5.	Commercial sugar 2%	-	-	-	-	-	-
6.	Commercial sugar 3%	-	-	-	-	-	-
7.	Glucose 2%	-	-	-	-	-	-
8.	Fructose 2%	-	-	-	-	-	-
9.	Lactose 2%	-	-	-	-	-	-
10.	Maltose 2%	-	-	-	-	-	-
11.	Glucose + Fructose 2 (1% each)	-	-	-	-	-	-
	SE (m) ±	0.30	0.70	0.07	0.12	0.10	0.13
	CD (0.05)	0.89	0.24	0.25	0.41	0.35	0.48

-Observation not recorded as no shoot produced rooted plantlet.

during growth of a culture. The usual practice is to adjust the pH of the medium within the range of 5.5 to 6.0 during the preparation of the medium. However, there are few reports on the effect of media pH on the growth of culture. Wali (1996) obtained best root and shoot growth at pH 5.5. The poor behaviour of pH 4.5 and 7.0 may be due to differential availability of various nutrients or due to some toxic effect attributed by high and low pH of the medium. In guava Amin and Jaiswal (1989b) observed that comparatively les acidic (pH 5.5 to 6.0) medium was better than more (pH 4.5 to 5.0) acidic medium for *in vitro* rooting.

Table 3. Influence of pH on *in vitro* rooting and shoot growth of Banana plantlets cv. Grand naine**.**

S/No	Treatments	Time taken for root initiation (days)	Cultures rooted (%)	No. of roots per shoot	Length of longest root (cm)	Length of shoot (cm)	No. of leaves per shoot
1.	pH 4.5	13.66	50.66 (45.36)	1.66	2.30	3.40	2.00
2.	pH 5.0	9.33	82.33 (65.14)	4.33	5.60	4.40	4.33
3.	pH 5.5	6.33	98.66 (83.70)	7.66	8.50	7.10	6.33
4.	pH 5.8	6.66	95.33 (78.68)	6.00	7.00	6.90	6.00
5.	pH 6.0	7.66	91.66 (73.26)	5.66	6.70	6.10	5.66
6.	pH 6.5	10.66	54.66 (47.66)	2.66	3.40	2.40	1.33
7.	pH 7.0	14.66	52.66 (46.51)	2.33	3.10	4.00	1.66
SE (m) ±		0.19	1.77	0.18	0.31	0.12	0.18
CD (0.05)		0.58	5.41	0.56	0.40	0.37	0.55

Table 4. Effect of supporting media on *in vitro* root and shoot growth of banana plantlets.

S/No	Treatments	Time taken for root initiation (days)	Cultures rooted (%)	No. of roots per shoot	Length of longest root (cm)	Length of shoot (cm)	No. of leaves per shoot
1.	Agar 0.8%	6.33	98.66 (83.32)	6.33	5.00	5.30	5.66
2.	Agar 0.4%	5.66	100.00 (90.00)	7.00	6.00	6.50	6.33
3.	Whatman No. 1 filter paper	-	-	-	-	-	-
4.	Ordinary filter paper	-	-	-	-	-	-
5.	Brown paper	-	-	-	-	-	-
SE (m) ±		0.05	4.65	0.09	0.11	0.06	0.10
CD (0.05)		0.18	14.86	0.37	0.47	0.23	0.41

- Observation not recorded as no shoot produced rooted plantlet.

Figure 2. Effect of different pH levels on *in vitro* rooting of banana plantlets.

Figure 3. Effect of supporting medium on *in vitro* rooting of banana plantlets.

Effect of supporting media

These results are in confirmation with those of Kitto and Young (1981) who reported increased response with the decrease in agar from 2.0 to 0.5%. Reports of Anderson (1980) support the result as they obtained best rooting by lowering the agar concentration.

Conflict of Interests

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

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Short Communication

Effect of *Verticillium fungicola* (PREUSS) HASSEBR inoculation in casing soil and conidial spray on white button mushroom *Agaricus bisporous*

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Dry bubble disease induced by *Verticillium fungicola* has been observed as an important disease of white button mushroom (*Agaricus bisporus*) in India. The symptoms produced on well differentiated fruit body are localized light brown depressed spots. The adjacent spots coalesce together to form irregular blotches. If the host pathogen infection is established before differentiation, sclerodermoid fruiting bodies appear on casing surface. Disease percent increased with the increased doses of inoculum whether inoculated in compost, casing or sprayed at the time of pinhead initiation. Fresh inoculum of pathogen (*V. fungicola*) was mixed on sorghum grains with sterilized casing soil. Inoculum of pathogen at 0.1, 0.2 and 0.3% per kg casing soil was mixed and applied at the time of casing. The negative effects was observed with increased doses of inoculum in casing. The introduction of *V. fungicola* at 3 g inoculum/kg casing soil delayed pinhead initiation thereby, more disease and less yield. Inoculum suspension prepared from actively growing mycelium when sprayed at the time of pinhead initiation drastically reduced the mushroom yield. Inoculum at 3 g/L resulted highest decrease in yield over control.

Key words: *Agaricus bisporus, Verticillium fungicola,* dry bubble, casing soil, inoculums.

INTRODUCTION

Mushroom, like any other crops, are attacked by several pests and diseases. Since they are grown indoors on specific substrates, their productivity and quality are adversely affected by a large number of biotic and abiotic

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factors. The most common biotic causes include parasitic and antagonistic fungi, bacteria, virus, nematodes, mites and insect pests. Mushrooms are grown on a specially prepared substrate (compost) which favours the selective growth of (*Agaricus bisporus*) mushroom mycelium. However, if composting is not carried out properly, weed moulds develop, some of which can reduce the yields significantly or even result in complete crop failure depending upon their severity and stage of appearance. This is more so under Indian conditions where significant proportion of the total mushroom production is contributed by seasonal growers who use unpasteurized compost prepared by long method composting (traditional method of preparation of compost) which harbour several parasitic fungi, antagonistic moulds and myceliophagous nematodes. Since the appearance of weed moulds indicates improper composting (unpasteurized), they are also known as indicator moulds.

Weed moulds compete with mushroom mycelium for space, water and nutrients, and are called competitor moulds. The main abiotic factors are temperature, relative humidity, CO_2 concentration, excess of moisture in compost and casing mixture, and presence of toxic chemicals in substrate or atmosphere. Most of the growers cultivate button mushrooms under natural climatic conditions taking one to three crops per year without any environmental control and insulation in ordinary rooms, abandoned poultry sheds and thatched huts. Poor hygienic conditions and lack of 'cook out' facilities also help in the perpetuation of various pathogens. Sometimes no facilities exist with the growers for measuring moisture, pH and N content of the compost, which are important quality parameters for ideal compost. Casing material used in India is sterilized with formaldehyde and in some cases not treated at all, which also introduces large number of pest and pathogen on compost. Presently, the scenario of mushroom cultivation in India is different from all the mushroom growing countries of the world with respect to substrates casing mixture and system of cultivation and so is the occurrence of disease.

Among the pathogenic fungi dry bubble disease caused by *V. fungicola* is the most dreaded disease of *Agaricus bisporous*. At some places heavy infection have been recorded resulting in failure of mushroom crops. The pathogen has been isolated from compost, casing soil, diseased button mushroom and mushroom house soil etc. Therefore, the present investigations were undertaken to understand the possible source of infection, resistant strains of *A. bisporous* and suitable management measures to minimize the losses. The symptoms produced under Haryana conditions on fully developed sporophores, are localized light brown depressed spots. Adjacent spots coalesce and form irregular brown blotches like those of bacterial blotches but lighter in colour and some what sunken at the center. Diseased caps shrink in blotched areas. If the infection had taken place during spawn run or before pinhead initiation, onion shaped deformed mushrooms produced instead of normal sporophore.

MATERIALS AND METHODS

The studies were carried out in the Mushroom Technology Laboratory (MTL), Department of Plant Pathology, CCS Haryana Agricultural University, Hisar.

Glassware and equipment

Glassware used in the present study were of Borosil. Polythene bags (30 × 45 cm), polypropylene bags (7.50 × 30 cm) is used for spawn production mushroom, and 500 ml empty glucose bottles were used for spawn and inoculum preparation.

Chemicals

The standard analytical grade chemicals were used in the present study.

Sterilization of glassware

Glasswares were sterilized at 180°C for two hours in a hot air oven.

Maintenance of culture

Pure cultures of *A. bisporus* and *V. fungicola* were maintained on PDA at ±23°C and ±20°C

Effect of pathogen inoculated in casing on dry bubble disease incidence

Fresh inoculum of the pathogen multiplied on sorghum grain was mixed with disinfected casing soil at 0.1, 0.2 and 0.3%. In check 2.0 g of M-140 spawn was mixed with disinfected casing mixture. This casing mixture was used in place of normal casing and five replicates of each treatment were kept.

Observation: At the time of picking diseased fruiting bodies were discarded and weight of the normal mushrooms was recorded and the data was expressed in percent decrease in yield over control.

Effect of inoculum spray on disease development

Culture of pathogen *V. fungicola* was prepared on broth media. Actively growing culture was harvested from the broth and different dilutions of inoculum sprays were prepared by using 1.0, 2.0 and 3.0 g of mycelial mat per litre of the sterilized distilled water. The suspension was homogenized in electric blender. Twenty milliliter of above inoculum (0.1, 0.2 and 0.3% concentration) dilutions per bag were sprinkled at pin head initiation condition of white button mushroom as per treatment and 20 ml sterilized distilled water was sprinkled per bag in control. On subsequent days normal water was sprayed for maintaining the desired humidity.

Observation: At the time of picking diseased fruiting bodies were discarded and the weight of normal mushrooms was recorded and the data were expressed in percent decrease in yield over control.

Table 1. Effect of *Verticillium fungicola* inoculation in casing on dry bubble disease.

S/N	Inoculum dose (g/kg casing soil)	Pin head initiation/ First picking (days)		Total yield (kg/100 kg compost)		Percent decrease in yield over control	
		2004-2005	2005-2006	2004-2005	2005-2006	2004-2005	2005-2006
1.	1	30/34	31/35	11.2	10.70	24.32	25.53
2.	2	34/38	34/38	9.5	8.79	35.81	38.83
3.	3	35/39	36/40	5.6	6.21	62.16	59.77
4.	Control	28/32	29/33	14.8	14.37	00.00	00.00
	CD at 5%			0.24	1.65		

Table 2. Effect of *Verticillium fungicola* spray on the yield of *A. bisporus*

S/N	Inoculum concentration (g/L)	Total yield (kg/100 kg compost)		Percent decrease in yield over control	
		2004-2005	2005-2006	2004-2005	2005-2006
1.	1	6.34	5.98	55.66	59.64
2.	2	4.20	3.94	70.62	73.41
3.	3	1.80	1.52	87.41	89.74
4.	Control	14.30	14.82	00.00	00.00
	CD at 5%	1.95	1.83		

RESULTS AND DISCUSSION

Effect of pathogen inoculated in casing on dry bubble disease

The production of button mushroom largely depends on a top dressing fungicides after the mushroom compost has been fully colonized with mushroom mycelium. To see the effect of casing material on infection with *V. fungicola*, that experiment was conducted. It was found that loss of mushroom yield increase with the increased doses of inoculum and up to 62.16% decrease in yield was recorded. It is presumed that yield loss may further increase with increase inoculum dose. The casing material as a source of pest inoculum was recognized by various scientists. Mantal (1973) recommended chemical sterilization of casing soil with formalin whereas Shandilya et al. (1976) advocated integrated sterilization of casing mixture that is, steam sterilization at 60°C for 1 h + Benlate 240 g/100 m^2. Jandaik and Gularia (2002) isolated *V. fungicola* from 12 month old spent compost and recommended proper sterilization of spent compost if used for casing (Table 1).

Effect of inoculum spray on disease development

Smith (1924) reported that mushroom beds inoculated by spraying or sprinkling with a suspension of *Mycogone spp.* spores in sterile water produced mushrooms with an external symptoms of wet bubble disease in addition a considerable number of sclerodermiod mushroom. It has been suggested that *Mycogon* attack the spawn and the parasitic hyphae mingle and grow side by side the mushroom. It is very improbable that the parasite could grow in content with the mushroom hyphae for any length of time as *Mycogone* produces enzymes which rapidly break down the hyphae of the mushroom.

In the present experiment first flush was almost normal, but in subsequent flushes of mushroom were more and more heavily infected until finally they were practically deformed with zero market value and as the bags became older all the mushroom were sclerodermoid. This may be due to broken strands of mycelium due to harvesting of mushroom. These broken ends frequently round off and form new button which are readily attacked by the pathogen (Table 2).

Conflict of Interests

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

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