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Table of Contents: Volume 12 Number 51, 21 December, 2017

ARTICLES

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Review

An overview of tomato fruit-ripening mutants and their use in increasing shelf life of tomato fruits

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The potential of tomato ripening mutants; rin, nor, alc, nr, gr, etc to prolong the shelf life of tomato cannot be overemphasized. Ripening mutants have gained a considerable attention in the last few years to extend shelf life. They have been characterized and examined for their potential utilization in lengthening shelf life of tomatoes. Mutant genes are therefore available to extend the shelf-life of tomato via breeding. These single recessive gene mutants mostly modify ethylene's downstream effects on specific biochemical processes related to fruit ripening. The fruits of these mutants are characterized by an absence of a ripening-associated ethylene burst and failure to ripen in the presence of exogenous ethylene. They are useful in research and breeding of cultivated tomatoes for postharvest quality. The effect is more pronounced in homozygotes where fruits do not develop normal colour. However, in the heterozygous form, several ripening characteristics exhibit levels that are intermediate between those of the wild and mutant parents and are able to produce fruits that have high shelf life than normal cultivars. Their discoveries have generated insights into ripening control and created new understanding of the primary ripening control mechanisms. Nonetheless, some scientists have argued that these mutant genes produce undesirable pleiotropic effects on other components of fruit quality. This is true in the homozygotes state, however in the heterozygote form, they develop acceptable colour for marketing, ripe naturally and exhibit extended shelf life too. Hence this offers an opportunity to exploit these genes to regulate tomato supply for longer period.

Key words: Ethylene, fruit ripening, ripening mutants, shelf life, tomato.

INTRODUCTION

Tomato (*Solanum lycopersicum* L. 2n=24*)* is one of the most widely cultivated and consumed vegetable crops in the world (Grandillo et al*.,* 1999) and is the second important vegetable crop after potato in the world (Saeed et al*.,* 2014). Tomatoes and tomato-based foods offer a wide variety of nutrients and lots of health-related benefits and can be eaten raw as fruits, salads or use as ingredient in the preparation of stews, soups, drinks and many other dishes (Alam et al., 2007). In Africa and especially Ghana where it is cultivated and consumed

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daily in every household, it constitutes a very important part of people's food (Wolff, 1999; Osei et al*.,* 2010). Wolff (1999) points out that because tomato is consumed daily in every household, consequently, it requires more money to purchase than any other vegetable. Production of tomato is thus an important source of income for most rural and peri-urban producers in most developing countries who are the main producers of this crop. Regardless of all the many benefits from the crop, many challenges beset its production. The challenges faced by tomato farmers and other stakeholders are attributed to a number of constraints in the tomato production, postharvest handling and marketing chain or a blend of them. A major dilemma in contemporary tomato production is postharvest handling. Tomato experiences great postharvest losses due to its natural perishability, precarious transportation, storage conditions and improper packaging. Kitinoja and Kader (2015) reported that the postharvest losses of fruits and vegetables in the developing countries account for almost 50% of the produce. Arah et al. (2015) further emphasized that postharvest losses in tomatoes can be as high as 25 to 42% globally. A fundamental limitation in fruit marketing is the premature ripening and softening during transportation. This predisposes the crop to rapid post-harvest softening and poor shelf life leading to great losses. Arah et al. (2015) indicated that these losses 'bring low returns to growers, processors and traders'. The authors again emphasized that this will result in loss of foreign exchange earnings in Ghana. Shelf-life is therefore an important quality trait that influences fresh tomato marketability, transportation and domestic use. Firm tomato fruits are also demanded by growers, shippers and processors to enable the fruit to withstand the rigors of shipping and reach the consumer in acceptable condition.

Several postharvest methods and treatments have shown some decline in fruit decay and weight loss in tomato. Such treatments include the use of gibberellic acid, calcium chloride, potassium and salicylic acid with chlorine (Mujtaba et al*.,* 2014). Other methods include packing in perforated polyethylene bag and kept at ambient condition. These methods are however, quite laborious and impracticable in farmers' fields. Additionally, advanced technique RNA interference can be used to down regulates the genes involved in ethylene biosynthesis and cell-wall degrading enzymes to extend the shelf life in tomato (Yogendra and Gowda, 2013; Carrari et al*.,* 2007). However, this involves genetic modification which is not supported by some countries due to lack of environmental safety and social acceptance of genetically modified crops. A high yielding tomato cultivar with high quality fruit and a long shelf life is one of the main goals in any fresh market tomato breeding program. Fresh-market tomatoes often have poor consumer quality, which is due in part to the marketing procedures used. Fruits are picked green and ripening is initiated by ethylene treatment for fresh market. This practice can produce satisfactory or acceptable fruit when tomatoes are picked mature-green, but often immature green fruits are also harvested. The immature green fruits will develop red pigmentation without acquiring the flavor, texture, and quality of a vine-ripe tomato (Kader et al*.,* 1977). This commercial practice is developed to facilitate tomato harvest, prolong fruit shelf life and minimize physical damage to the fruit during handling. The regulatory events controlling fruit ripening have greatly benefitted from the availability of natural ripening mutants. Use of the ripening mutant genes in the heterozygous condition offers the possibility of picking fruit at early stages of red pigment development, thereby ensuring harvest of mature fruit, and yet having adequate shelf life (Kopeliovitch and Rabinowitch, 1979; McGlasson et al*.,* 1983; Tigchelaar et al*.,* 1978). The present review discusses commercially used ripening mutants as potential germplasm for prolonging shelf-life of cultivated tomato and emphasizes the characteristic expression and effects of *rin*, *nor* and *alc*. It also highlights the prospects and problems associated with using the mutants.

TOMATO FRUIT DEVELOPMENT

Tomato fruit's development is initiated by fertilization (Picken, 1984; Gillaspy et al*.,* 1993). Fruits can be formed when the yellow flowers on the tomato plants are fertilized. As soon as they are fertilized, the flowers develop into small green globes that become visible at the base of the blossoms and ultimately become mature fruits (tomatoes). Tomato plants possess both the male and female reproductive parts often referred to as monoecious. The male reproductive part is structured in such a way that inside each flower, pollen from the male stamen is able to pollinate the female pistil thereby ensuring fertilization. Though this does not happen in every single flower on each plant, the process can be aided by agents such as insects (eg. Bees), wind and Man. The insects find themselves inside the flowers for nectar and move the pollen from one place to the other. Man and wind also ensure pollination by shaking the plants in an effort that causes the pollen grains to disperse.

A single tomato branch can have many or few yellow flowers with the prospective flower first appearing on the end of the branch as a small tightly closed bud contained by outer leaves designated as sepals, also collectively known as the calyx. Right inside the calyx, the yellow petals, or the corolla, of the tomato flower develop around its reproductive organs composed of the (pistal) that houses the bright green carpels where fruit formation takes place following fertilization. Once fertilized, the yellow petals wither and fall off to make way for the developing fruit. That is, the flower begins to age, a process called senescence, shedding its petals and female reproductive organs, revealing the tiny green tomato still attached to the sepals. At this stage, unfertilized flowers are however, excluded by the plant and separated just below the sepals. Even though flowers in a cluster do not develop and mature at the same rate, it is common to see unopened buds, mature blossoms and developing tomatoes on a single branch at the same time. The ovary wall is transformed into the pericarp as soon as fertilization is successful. The pericarp can be divided into three different structures: exocarp, mesocarp, and endocarp. The external exocarp is made up of a cuticle layer and the skin. The cuticle layer thickens as the fruit ages. The skin however, contains an epidermal cell layer and a collenchymatous tissue (three to four layers), in which starch accumulates and few plastids are retained (Esau, 1953; Varga and Bruinsma, 1986; Joubès et al*.,* 2000; Lemaire-Chamley et al*.,* 2005). The mesocarp, the intermediate layer, is a parenchymatous tissue formed by big cells with large vacuoles (Joubès et al*.,* 2000; Lemaire-Chamley et al*.,* 2005; Mintz-Oron et al*.,* 2008). The cells of the mesocarp commonly undergo six to eight rounds of DNA duplication (endocycles) reaching ploidy levels of up to 512C (Bourdon et al*.,* 2010) and are reminiscent of the palisade cells of leaves (Gillaspy et al*.,* 1993) since they contain several chloroplasts, the organelle where photosynthesis occurs and produces up to 20% of fruit photosynthates, whereas the rest of photo assimilates are imported from source leaves (Hetherington et al*.,* 1998).

Tomato fruit development can be classified into four different phases. The first, dubbed the fruit set phase, corresponds to the stage where the development of the ovary either proceeds or aborts. The initiation of ovary development normally depends on the success of the pollination and fertilization. The ovary begins to develop into the fruit once the ovules are fertilized. During this stage, cell division and enlargement result in slow growth. Plant hormones such as gibberellins and auxins have been shown to play an important role in fruit set. These hormones when artificially applied can trigger the development of parthenocarpic (seedless) fruits (Gustafson, 1960; Gillaspy et al*.,* 1993). Again, this phase is described by rapid cell division, thereby resulting into a progressive increase in pericarp cell number.

The rate of cell division decreases barely two weeks after pollination marking the end of phase one. At this stage the fruit is about 1 cm in diameter. The subsequent phase of fruit development is characterized by a period of extensive cell division of the fruit tissue that generally takes between seven and ten days after fertilization depending on genotype (Varga and Bruinsma, 1966; Mapelli and Lombardi, 1982; Bohner and Bangerth, 1988). All through this stage, fruit growth depend on cell expansion and leads to a significant increase in weight. Cell expansion coincides with endo reduplication (Bergervoet et al*.*, 1996). By the end of this stage fruits have a diameter of around 2 cm. Throughout the third phase, the fruit enters the mature green (MG) stage (Ho and Hewitt, 1986; Giovannoni, 2004; Czerednik et al*.,* 2012) and attains its final size, which varies greatly among cultivars and is very susceptible to environmental influences (Chevalier, 2007). Most fruit growth is associated with the subsequent expansion phase during which cells undergo substantial endoreplication resulting in the production of large high ploidy cells with a diameter of more than 0.5 mm and up to 512C DNA content (Cheniclet et al*.,* 2005). This growth phase is driven by the accumulation of water in the vacuole. At the end of the cell expansion period, the tomato fruit reaches its final size and contains mature seeds. Roughly two days after reaching the mature green (MG) stage, the tomato fruit undergoes an extensive metabolic reorganization, which marks the beginning of the fruit ripening process (Ho and Hewitt, 1986). The mature fruit then undergoes the last phase of fruit development, known as ripening. Two main phases can be distinguished, which are referred to as the breaking (BR) and the ripening (RR) stages. From a botanical point of view, the tomato fruit is a berry, which can be bi- or multilocular. The septa of the carpels divide the ovary and the fruit into two or more locules. Seeds develop attached to the placenta, a parenchymatous tissue, which becomes gelatinous and fills the locular cavities during fruit development and maturation (Grierson and Kader, 1986; Ho and Hewitt, 1986; Bertin, 2005; Mintz-Oron et al*.,* 2008).

TOMATO FRUIT RIPENING AND RIPENING MUTANTS

Ripening is a phenomenon accountable for producing fruit that are eye-catching for consumption. Ripe fruits are a great source of energy, minerals, vitamins, carotene and antioxidants. Ripening is studied not only for its role in nutrition, but also its unique and complex developmental process requiring the well-coordinated regulation of numerous biochemical pathways. Study of fruit ripening is therefore valuable not only for practical agricultural purposes but also to better understand the regulation and transposition of plant developmental programs. Ripening is said to be a deteriorating process involving senescence and the general breakdown of cells. Fruit ripening is a sophisticatedly orchestrated developmental process, unique to plants, that results in major physiological and metabolic changes, ultimately leading to fruit decay and seed dispersal. Because of their strong impact on fruit nutritional and sensory qualities, the ripening associated changes have been a matter of sustained investigation aiming at unraveling the molecular and genetic basis of fruit ripening. All biochemical, molecular, physiological and structural modifications associated with ripening are tightly orchestrated at the genetic level, enabling the control of appearance, aroma and flavour. Ethylene is required for fruit ripening and plays other important roles in plant growth and development (Barry, 2007). Ripening in the cultivated tomato comprises a series of biochemical and physiological events, including softening, pigment change, development of flavor components, autocatalytic ethylene production, and climacteric respiratory behavior, which together make ripe fruits. During ripening, tomato increases their ethylene level and subsequently undergoes various physiological changes. These changes occur simultaneously and are caused by the highly synchronized expression of numerous genes at the onset of ripening. Ethylene is rapidly produced at the breaker stage of the fruit and drives a series of reactions that together define the fruit ripening process. Generally, ripening involves softening of the fruit tissue, conversion of starch to sugar, accumulation of secondary metabolites affecting appearance, taste and aroma (Seymour, 1993). Polygalacturonase (PG) is the most important softening enzyme in tomato. It is absent in green fruit and accumulates in large quantities during ripening. The presence of this enzyme is correlated with the onset of cell wall degradation. Natural ethylene synthesis begins before PG appears and exogenous ethylene causes the accumulation of this enzyme in mature fruit (Grierson and Kader, 1986).

The process that leads to tomato ripening can be further divided into mature green stage, breaker stage and red ripe fruit stage. The mature green stage refers to the final-sized fruit containing mature seeds before initiation of ripening. The breaker stage corresponds to the beginning of the ripening program in the fruit, and is characterized by the first visual sign of ripening (orange color at the base of the fruit) and production of high levels of ethylene. Numerous genes associated with ripening begin to be expressed at high level at this stage (Alba et al*.,* 2004). The red ripe fruit stage corresponds to the fruit having completed the ripening program. Again the time required to reach red ripe fruits from BK is typically three to ten days, depending on the variety.

Although numerous ripening-associated traits have been shown to be influenced by ethylene, the identification and characterization of several naturally occurring ripening mutations have revealed another layer of regulation acting upstream of ethylene. Ripening mutants particularly alcobaca (*alc*), non-ripening (*nor*), never ripe (*nr*) and ripening inhibitor (*rin*) have gained a considerable attention in the last few years to prolong shelf life of tomato. These genes generally inhibit ethylene synthesis and or modify ethylene's downstream effects on specific biochemical processes related to fruit ripening. The fruits of these mutants are generally characterized by an absence of a ripening-associated ethylene burst, and an inability to ripen in the presence of exogenous ethylene. They are useful in research and breeding of cultivated tomatoes for postharvest quality. Kopeliovitch et al. (1979) have used several ripening gene mutants such as *alc*, *nor*, *nr* and *rin* to develop lines and cultivars with delayed ripening through disruption of the ethylene signalling pathway. Rodriguez et al. (2010) also explored the inheritance of fruit quality traits such as fruit shelf life in some tomato crosses using an Argentinean cultivar and a ripening mutant (*nor*). The mutant alleles *rin*, *nor* and *alc* generate a somewhat similar extended shelf life phenotype in heterozygous plants at these loci and have different modes of action.

CHARACTERISTIC EXPRESSION AND EFFECTS OF *rin, nor* AND *alc*

Ripening inhibitor (*rin*) is a regulator of ripening that directly influences many ripening- associated processes in a specific pattern. The *rin* mutant appeared in an F4 breeding line developed by H. M. Munger at Cornell University. The recessive gene altered several aspects of ripening as fruits did not ripen fully, turned yellow and softened very slowly (Robinson and Tomes, 1968). It encodes a genetic regulatory component necessary to trigger climacteric respiration and ripening-related ethylene biosynthesis. The *rin* gene is located on tomato chromosome 5 and belongs to the MADSBOX family of transcription regulators which is known to play essential roles in a variety of plant development processes (Riechmann and Meyerowitz, 1997). The name MADS is an abbreviation of the four founding members of the family: Mcm1 from *S. cerevisiea* (Passmore et al*.,* 1989), AGAMOUS from *Arabidopsis thaliana* (Yanofsky et al*.,* 1990), DEFICIENS from *A. majus* (Sommer et al*.,* 2000) and Serum Response Factor (SRF) from Homo sapiens (Norman et al*.,* 1988). MADS box genes encode DNA-binding proteins involved in many developmental processes in yeast, insects, nematodes, lower vertebrates, mammals and plants (Becker and Theissen, 2003; Messenguy and Dubois, 2003). In this regard, *rin* is a comprehensive ripening regulator explaining both the severe ripening inhibition of the mutation and its utility in coordinately slowing virtually all ripening processes in hybrid *Rin*/*rin* fruit predominant in current fresh market tomato production. Tomato plants homozygous for the ripening-inhibitor (*rin*) mutation have fruits that fail to ripen. Moreover, *rin* plants show enlarged sepals and loss of inflorescence determinacy. Two tandem MADS-box genes (LeMADS-RIN and LeMADS-MC) were revealed at positional cloning of the *rin* locus. Their expression patterns suggested roles in fruit ripening and sepal development for LeMADS-RIN and LeMADS-MC respectively. The *rin* mutation alters expression of both genes. The *rin* mutant exhibits ethylene sensitivity, including the seedling triple response, floral abscission, and petal and leaf senescence. Nevertheless, *rin* fruit do not ripen in response to exogenous ethylene, yet they display induction of at least some ethylene responsive genes, indicating retention of fruit ethylene sensitivity. The *rin/rin* homozygous plant produces fruit that never fully ripen and have much firmer fruit with a significantly longer shelf life. At maturity, fruits are green and later turned into bright yellow, retarded ripening.

In the heterozygous condition *rin*/+ plants produce fruit with near normal fruit colour and flavour, and shelf life that is intermediate between *rin/rin* and +/+ (wild type) plants. *Rin/rin* lines generally have very low fruit sugars. They however, vary in sugar levels between *rin/rin* lines and +/+ lines. Lycopene levels in rin/+ hybrids is a little lower than wild type. Ripening is a little slower with *rin*/+ hybrids. Tomatoes heterozygous for the *rin* allele stay firm and ripen over a lengthened period. This is most probably due to reduced levels of functional RIN protein which allows industrial-scale handling and expanded delivery and storage opportunities.

The non-ripening (*nor*) gene was identified in an introduction, 'Italian Winter,' by E.A. Kerr at Horticultural Research Institute, Ontario. The *nor* gene is located on tomato chromosome 10 and belongs to the NAC family of transcription factors whose members play important regulatory roles in numerous developmental programs (pathways) (Olsen et al*.,* 2005). The NAC family is the largest plant-specific family of transcription factors with more than 100 members identified in *A. thaliana* (Riechmann et al*.,* 2000). NAC genes are named for their 18 founding members*:* Petunia *hybrida* NAM and *A. thaliana* ATAF1/2 and CUC2 genes (Souer et al*.,* 1996; Aida et al*.,* 1997). The *nor* gene is an unrelated transcription factor that also serves as a master regulator of fruit ripening in tomato. The *nor/nor* homozygous plant has a very similar phenotype to *rin/rin,* and *nor*/+ hybrids also have much restored color and flavor with extended shelf-life. *Nor* prevents normal fruit ripe (non-pigmentation, non-softening and crack resistance in fruits). This mutation is often used in the heterozygous form to create long shelf life of fruits. The non-ripening phenotype associated with the *nor* mutation suggests that many biochemical pathways are influenced by this gene.

The cultivar Alcobaca from Portugal possessing slow ripening gene *alc* was discovered in the 1960'sdescribed as a tomato with prolonged fruit storage life (Almeida, 1961) and potato type leaf (Lu et al*.,* 1995, Robinson and Tomes, 1968). Alcobaca genes in its homozygous form is noted to obstruct or significantly slow down a wide range of processes associated with ripening of tomato fruit leading to a noticeably extended shelf life (Kopeliovitch et al*.,* 1979; Lobo et al., 1984; Mutschler, 1984b; Tigchelaar and Rios, 1989; Lu et al*.,* 1995; Ignatova et al., 1999; Dhatt, 2001; Garg, 2006) but inferior flavour (Kopeliovitch et al*.,* 1980, 1982) and poor colour development (Sink et al*.,* 1974; Kopeliovitch et al*.,* 1980; Lobo et al*.,* 1984). However, in plants heterozygous for these alleles, several ripening characteristics exhibit levels intermediate between those of the wild and mutant parents and produce fruits having shelf life many times greater than normal cultivars (Kopeliovitch et al*.,* 1979; Mutschler et al*.,* 1992; Dhatt, 2001; Kitagawa et al., 2005; Garg, 2006), besides developing acceptable colour (Kopelivitch et al*.,* 1981; Mutschler, 1984b; Gavrish and Korol, 1991) and flavour attributes (Kopeliovitch et al*.,* 1982; Agar et al*.,* 1994). This semi-climacteric mutant causes a ripening syndrome characterized by attenuated respiratory activities and ethylene production, delayed softening of the fruit, low PG activity and extended shelf life (Mutschler, 1984b). Kopeliovitch et al. (1980) found that the final colour of mutant fruits picked at mature green stage, breaker stage and two weeks post breaker stage was yellow, orange and light red, respectively.

UTILIZATION OF *rin, nor* AND *alc* ALLELES

Fruits of tomato are only available for a short time. Excess production of tomato during these short periods leads to a saturated market and low prices. These periods can be followed by scarcity and high prices. Mutant genes such as alcobaca (*alc*), ripening inhibitor (*rin*), and non-ripening (*nor*) in heterozygous form extend shelf-life and take more time to go from mature green to red ripe as compared to normal genotypes (Buescher et al*.,* 1976; Kopeliovitch et al*.,* 1979; McGlasson et al*.,* 1983; Nguyen et al., 1991; Mutschler et al*.,* 1992; Agar et al*.,* 1994; Lu et al*.,* 1995; Dhatt, 2001; Kitagawa et al*.,* 2005) and develop acceptable color (Sink et al*.,* 1974; Ng and Tigchelaar, 1977; Hobson, 1980; Kopeliovitch et al*.,* 1981; Mutschler, 1984; Lu et al*.,*1994) and flavor attributes (Kopeliovitch et al*.,* 1982; Agar et al*.,* 1994). Fruit of F1 hybrids carrying these mutant alleles take longer to transition from mature green to red ripe stages as compared to normal genotypes (McGlasson et al*.,* 1983; Nguyen et al*.,* 1991; Dhatt, 2001). Sinha et al. (2014), in their work on tomato shelf life, evaluated twenty four F7 RIL'S developed from the cross L121 X Vaibhav for high shelf life. L121 is a parent having *alc* gene is able to prolong shelf-life but has poor agronomic character. The evaluation of F7 RILs resulted in the identification of tomato RILs with high shelf life. Among F7 RILs, parents and checks, the maximum shelf life was observed in RIL 7-3, RIL 110-2 (110 days), followed by RIL 102-1 and RIL 182-4 (100 days) with an average shelf life of the F7 RILs were 63 days.

According to Giovannoni (2007), the discoveries of fruit ripening mutants have yielded insights into ripening control. He further mentioned that these findings have produced new understanding of the primary ripening control mechanisms which include transcription factors such as those encoded by the RIPENING- INHIBITOR (RIN) MADS-box and COLOURLESS NON-RIPENING (CNR) SPB-box genes. These are actually necessary for the progression of virtually all ripening processes. They have also facilitated the elucidation of downstream signal transduction components that impact the hormonal and environmental stimuli that coordinate and modulate ripening phenotypes. He demonstrated in his report that physiologically characterized single gene tomato ripening mutants, which in some cases have been available for decades, have recently become accessible and useful at the molecular level as the genomics infrastructure for tomato has expanded.

Lavy-Meir et al*.* (1989) also reported that tomato ripening mutants and their hybrids showed resistance to *Botrytis cinerea.* Their work examined the resistance of mutant fruits to infection by *B. cinerea,* one of the main pathogens of tomato fruit by studying differences in conidia germination, infection and lesion development among normal, mutant and hybrid fruits. Yasuhiro (2016) further confirmed in his report or review that the identification of ripening mutants particularly key regulatory gene *rin* in tomato has opened new horizons in our understanding of fruit ripening. He also reported that the *rin* mutant removes the transcriptional activation activity from the protein thereby preventing the up-regulation of genes required for ripening (Ito et al*.,* 2008). Additionally, his review revealed that *rin* regulates other transcription factors involved in fruit ripening, including *nor* and *cnr*. Ethylene mediates the induction of ripening-associated genes under the control of the ripening regulating transcription factors, and the ethylene signalling pathway also enhances the expression of RIN, FUL1, and NOR in a positive feedback loop (Fujisawa et al*.,* 2013). Understanding of ripening regulation, especially the crucial role of MADS-box transcription factors, have expanded scientists ability to control ripening-associated metabolic characteristics, thereby providing breeding methods for fruits with better taste, aroma, nutrition, and shelf life. The author further reported that apart from biological interest in ripening regulation, breeding programs have targeted the RIN locus because the RIN/rin heterozygous genotype produces red-ripe fruits with a remarkably extended shelf life (Kitagawa et al*.*, 2005). The heterozygous genotype has also been proposed for use in developing tomatoes with low levels of allergens (Kitagawa et al*.,* 2006).

PROBLEMS AND PROSPECTS OF TOMATO RIPENING MUTANTS

The remarkable features of *rin, nor* and *alc* mutants are their extended shelf life beside quality parameters (except colour) comparable with the normal fruit (Kopeliovitch et al., 1979; MutscWer et al., 1984b; Lu et al., 1994; Hobson 1980; McGlasson, 1983; Lobo et al., 1984; Lobo et al., 1991; Mutschler et al., 1992). Mutant genes such as *rin, nor* and *alc* can be used to extend the shelf life of tomato (Leal and Tabim, 1974; Robinson and Tornes, 1968; Tigchelaar et al*.,* 1973). The fruits of these mutants are elite by an absence of a ripening-associated ethylene burst, and an inability to ripen in the presence of exogenous ethylene. This phenotype is described as a failure to reach ripening competency, a developmentally regulated stage in which a fruit becomes responsive to ethylene. These mutant genes not only delay the normal process of ripening but also have undesirable pleiotropic effects on other components of fruit quality (Kovacs et al*.*, 2009; Matas et al*.,* 2009; Mutschler et al*.,* 1992; Thompson et al*.,* 1999; Tigchelaar et al*.,* 1978). The abnormal pattern of fruit colour development in the homozygous form has restricted their use at commercial levels (Robinson and Tomes, 1968; Tigchelaar et al*.,* 1973; Lobo et al*.,* 1984). For instance, the non-ripening (*nor*) gene repress the normal ripening of tomato fruit. The molecular mechanism of fruit ripening regulation by the *nor* gene is however, unclear. Unfortunately, in homozygous state these genes do not develop normal colour (Kopeliovitch et al*.,* 1979). Even exogenous application of ethylene is not helpful in stimulating colour development (Tigchelaar et al*.,* 1978). Some efforts employed by some scientists to overcome the problem associated with the homozygous state of the ripening mutant genes is by cross combinations with desired genotypes for colour improvement. Trinklein and Lambeth (1976) proposed incorporation of uniform ripening and colour enhancing genes to improve colour.

Nguyen et al*.* (1991) and Dhatt (2001) demonstrated that *rin, nor* and *alc* alleles in heterozygote form nonetheless, developed acceptable colour for marketing. This was also confirmed by Mutschler (1984), Lu et al*.* (1994) and Seroczynska et al*.* (1998) who reported that the fruits of hybrids between normal homozygotes (+/+) and mutant homozygotes (*nor/nor, rin/rin and alc/alc*) do ripen naturally and exhibited extended shelf life too. Hence, this offers an opportunity to exploit these genes to regulate tomato supply for longer period. The commercial use of *rin, nor* and *alc* mutants has been made in many countries. In Australia, several varieties have been developed using ripening mutants. For instance, 'Red Centre' (HRAS 87-70 x *rin-HRAS* 81-85) and 'Juliette' (79T-I x *rin-795054-1)* hybrids having 40 days shelf life at 20°C temperature have been released (Nguyen et al*.,* 1991; Nguyen 1994). The *nor* gene has been exploited by Gavrish and Bogdanov (1992) in Russia by developing 'Vasilisa' hybrid, 'Changline' an outstanding *nor* hybrid with exceptional shelf life was also released in China (Lu et al*.,* 1994). The F1 between S 15 x *110r* was registered as Rafal' in Poland, which has storage life of 77 to 99 days. It also provided 2 to 4 weeks delay in last harvest along with high firmness, acceptable colour and good quality (Seroczynska et al*.,* 1998). Likewise in the USA (Suslow and Cantwell, 1997) and India, ripening mutant genes have been successful at the commercial level (Dhatt, 2001). The alcobaca (*alc*) gene, a mutant involved in the ripening process of tomato plant fruits, have allowed an understanding about some effects that it causes on the quality and post-harvest conservation traits of tomatoes. The effects of the *alc* allele on several plant and fruit traits were studied by Mutschler et al*.* (1992), Flori (1993), Flori and Maluf (1994), Resende (1995), Souza (1995) and Freitas (1996).

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Correlation analysis for grain yield and other agronomic parameters for 90 single crosses hybrid maize evaluated in three agrological zones in Ghana

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A trial was conducted to determine the correlation between grain yield and agronomic parameters of 90 F_1 early maturing maize hybrids in 2012 in Fumesua, Ejura and Kpeve; representing the Forest, Forest-Savannah Transition and Coastal- Savannah Transition zones of Ghana, respectively. The objective of the work was to determine the correlation between grain yield and other agronomic parameters of maize across three locations. Randomized Complete Block Design (RCBD) with two replicates was used for each location. Results from the correlation analysis revealed that grain yield was significantly and positively correlated with plant height (r = 0.633), cob length (r =0.610) ear height (r =0.410), and cob diameter (r = 0.401). However, there were nonsignificant correlation between grain yield and days to silking. Nevertheless, among agronomic traits, ear height, plant height seed length, seed diameter, cob length and cob diameter were positively and significantly correlated, indicating that increase in any one of these traits could lead to increase in the other. It was recommended that hybrids that showed the highest correlation with grain can be selected to improve grain yield.

Key words: Maize, correlation, genotype, hybrid, adaptability, yield, agroecology.

INTRODUCTION

Maize (*Zea mays* L.) is the most widely grown cereal in the world, and it is the third most important cereal crop after wheat and rice which serves as a primary staple food in most developing countries (Khalil et al., 2011; Badu-Apraku et al., 2010; Obeng-Bio, 2010). The maize plant has wider adaptability hence can be cultivated in different growing conditions from latitude 58°N to 40°S, below sea level and at altitude higher than 3000 m, and in areas with rain-fall of about 250 to 5000 mm per annum and with growing season ranging from three months to about 13 months (Golam et al., 2011).

Its high yield potential, wide adaptability, relative ease

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Table 1. Description of the test environment used in the trial.

Location	Latitude	Longitude	Altitude	Rainfall (mm)	Ecology
Fumesua	6° 43'N	1° 36W	228	142.4	Forest
Ejura	7° 24'N	1° 21E	229	117.4	Forest Savanna Transition
Kpeve	3° 20'N	0° 17E	69	121.5	Coastal Savanna Transition

Rainfall data from April to August (2012).

of cultivation, processing, storage and transportation has increased its potential for combating food security challenges posed by population increase and changes in climatic conditions due to global warming in West and Central Africa (Badu-Apraku et al., 2010).

Maize is grown in approximately 25 million hectares in Sub-Saharan Africa, largely by subsistence farmers that produced 38 million tons in 2005 to 2008, primarily for food (Smale et al., 2011). From 2005 to 2008, maize represented an average of 27% of cereal area, 34% of cereal production and 8% of the value of all primary crop production (FAO STAT, 2014; Smale et al., 2011).

In Ghana, maize is the highest ranking cereal in terms of production and consumption followed by rice (Twumasi-Afriyie et al., 1992). The domestic demand is growing because it serves as a major source of daily calories and dietary protein for most people who are under privileged, since poverty makes it difficult for such people to afford meat (MiDA, 2006).

According to a MOFA (2006) annual report, maize accounted for 50 to 60% of total production area of cereals with average yield approximately at 1.6 metric tons per hectare, but yields as high as 4.5 to 5.0 metric tons per hectare can be realized by farmers using improved seeds and good management practices.

MATERIALS AND METHODS

Research location

The experiment was conducted in three agro-ecological zones of Ghana. The locations where the experiment was conducted were different in mean seasonal rainfall (Table 1). Fumesua lies in the Forest ecology zone of Ghana. Ejura lies in the Forest –Savannah Transition ecology and Kpeve lies in the Coastal-Savanna Transition. The three experimental sites experience a bimodal rainfall pattern. The major season stretches from April through July, and the minor season from August to November (Table 2).

Research materials

Experimental design

The ninety F_1s were constituted into a hybrid trial and planted in a random complete block design at each of the sites. Prior to planting, the site was thoroughly prepared with plough and harrow using tractor. Each entry was planted in a one row of 5 m, spacing between hills of 0.45 m and spacing between rows of 0.75 m with two replications at each of the three evaluation sites. Each plot in the trial contained 11 hills, and each row contained 22 plants to obtain a target plant density of approximately 60,000 plants ha-^{1}.

Crop husbandry

Pre-emergence and post emergence chemical weed control was done with an application of Gramoxone and Atrazine respectively. Hand weeding was also done when necessary to control weeds during the growing period. NPK 15-15-15 fertilizer was applied at the rate of 30 kg N ha^{-1}and 60 kg P_2O_5 ha^{-1} as basal fertilizer at two weeks after planting and top-dressed with additional N at 60 kg N ha-^{1} at 4 weeks after planting. The trials were conducted under rain-fed condition, and other management practices were done according to the recommendations of the specific areas.

Data collection

Data were collected on the following parameters prior to harvesting:

Days to 50% tasseling

This was calculated as the number of days from the date of sowing to the day at which 50% of plants in a plot showed full tassel emergence.

Days to 50% silking

The number of days from the date of sowing to the day on which 50% of the plants in a plot showed complete silk emergence.

Plant height

The height of five randomly selected plants in a plot were measured in centimeter with a graduated measuring stick from the ground level to the node bearing the flag leaf and averaged.

Ear height

The ear heights of the five previously selected plants in each plot were measured in centimeters from ground level to the node bearing the uppermost ear and averaged.

Cob length

The length of the cob was measured in centimeters using Vernier caliper (from the base of the ear to the tip). Five cobs were chosen at random from each plot and averaged.

Cob width

The widths of five randomly selected cobs were measured in centimeters as the thickness of the ear using Vernier caliper. Ten cobs were chosen at random from each plot and averaged.

Table 2. List of 90 single cross hybrid maize evaluated at three locations in southern during 2012 growing season.

Entry no.	Code	Entry name	Entry no.	Code	Entry name
1	P1	TZEI-9 × TZEI-12	46	P46	TZEI-19 × TZEI-46
2	P2	TZEI-24 × TZEI-23	47	P47	TZEI-36 × TZEI-20
3	P3	TZEI-48 × TZEI-20	48	P48	TZEI-14 × TZEI-15
4	P4	TZEI-4 × TZEI-2	49	P49	TZEI-12 × TZEI-9
5	P5	TZEI-3 × TZEI-1	50	P50	TZEI-34 × TZEI-46
6	P6	TZEI-2 × TZEI-19	51	P51	TZEI-1 × TZEI-19
7	P7	TZEI-9 × TZEI-11	52	P52	TZEI-22 × TZEI-48
8	P8	TZEI-41 × TZEI-47	53	P53	TZEI-33 × TZEI-19
9	P9	TZEI-41 × TZEI-30	54	P54	TZEI-39 × TZEI-22
10	P10	TZEI-11 × TZEI-15	55	P55	TZEI- 9 × TZEI-10
11	P11	TZEI-46 × TZEI-34	56	P56	TZEI-25 × TZEI-27
12	P12	TZEI-10 × TZEI-11	57	P57	TZEI-22 × TZEI-20
13	P13	TZEI-32 × TZEI-5	58	P58	TZEI-14 × TZEI-16
14	P14	TZEI-38 × TZEI-35	59	P59	TZEI-22 × TZEI-45
15	P15	TZEI-12 × TZEI-13	60	P60	TZEI-19 × TZEI-48
16	P16	TZEI-35 × TZEI-19	61	P61	TZEI-33 × TZEI-2
17	P17	TZEI-39 × TZEI-34	62	P62	TZEI-12 × TZEI-20
18	P18	TZEI-22 × TZEI-20	63	P63	TZEI-22 × TZEI-18
19	P19	TZEI-39 × TZEI-36	64	P64	TZEI-28 × TZEI-14
20	P20	TZEI-45 × TZEI-47	65	P65	TZEI-2 × TZEI-34
21	P21	TZEI-36 × TZEI-34	66	P66	TZEI-34 × TZEI-22
22	P22	TZEI-17 × TZEI-16	67	P67	TZEI-36 × TZEI-38
23	P23	TZEI-11 × TZEI-12	68	P68	TZEI-2 × TZEI-22
24	P24	TZEI-13 × TZEI-17	69	P69	TZEI-27 × TZEI-19
25	P25	TZEI-35 × TZEI-16	70	P70	TZEI-18 × TZEI-46
26	P26	TZEI-25 × TZEI-23	71	P71	TZEI-36 × TZEI-35
27	P27	TZEI-48 × TZEI-45	72	P72	TZEI-14 × TZEI-17
28	P28	TZEI-42 × TZEI-47	73	P73	TZEI-27 × TZEI-14
29	P29	TZEI-31 × TZEI-18	74	P74	TZEI-36 × TZEI-33
30	P30	TZEI-9 × TZEI-15	75	P75	TZEI-31 × TZEI-7
31	P31	TZEI-47 × TZEI-34	76	P76	TZEI-22 × TZEI-46
32	P32	TZEI-17 × TZEI-15	77	P77	TZEI-42 × TZEI-30
33	P33	TZEI-39 × TZEI-30	78	P78	TZEI-34 × TZEI-7
34	P34	TZEI-41 × TZEI-46	79	P79	TZEI-33 × TZEI-3
35	P35	TZEI-30 × TZEI-47	80	P80	TZEI-46 × TZEI-47
36	P36	TZEI-11 × TZEI-9	81	P81	TZEI-41 × TZEI-22
37	P37	TZEI-27 × TZEI-9	82	P82	TZEI-33 × TZEI-46
38	P38	TZEI-36 × TZEI-22	83	P83	TZEI-25 × TZEI-14
39	P39	TZEI-13 × TZEI-10	84	P84	TZEI-34 × TZEI-3
40	P40	TZEI-36 × TZEI-39	85	P85	TZEI-19 × TZEI-18
41	P41	TZEI-45 × TZEI-34	86	P86	TZEI-48 × TZEI-16
42	P42	TZEI-12 × TZEI-15	87	P87	TZEI-10 × TZEI-12
43	P43	TZEI-30 × TZEI-31	88	P88	TZEI-18 × TZEI-26
44	P44	TZEI-4 × TZEI-3	89	P89	TZEI- 24 × TZEI-12
45	P45	TZEI-23 × TZEI-15	90	P90	TZEI-38 × TZEI-36

Grain yield

Grain yield kg ha^{-1} was calculated for every entry from the data of harvested ear weight per plot using the following formula:

Grain yield (kg ha^{-1}) was calculated as = Harvested ear weight (kg plot-1) × (100-MC) × 0.8 × 10,000/ (100-15) × 3.75 m^2 (at 15% moisture).

Seed length

The length of ten randomly selected seeds were measured in centimeters using Vernier caliper and averaged.

Seed diameter

The widths of ten randomly selected seeds were measured in centimeters as the thickness of the seed using Vernier caliper and averaged.

Data analysis

The Analysis of Variance (ANOVA) according to Steel and Torrie (1980) for grain yield and agronomic parameters was conducted using Statistical Analysis System version 9.2 (SAS, 2003). Least significance difference test ($p \leq 0.05$) was used to determine the level of significance among measured parameters. Pearson coefficients of correlation were calculated using the hybrids' least square means for all parameters to determine associations among these parameters. Correlation coefficients ranged in values between -1 and +1; a perfect negative relationship and a perfect positive relationship respectively

RESULTS AND DISCUSSION

Mean performance of the genetic materials evaluated

Mean performance of the crosses are presented in Table 4. Mean grain yield was 4598 kg ha^{-1} with yield ranging from 1058.4 kg ha^{-1} (TZEI-45 × TZEI-47) to 6296 kg ha^{-1} (TZEI-36 × TZEI-39). The mean performance for plant height showed that differences among genotypes were highly significant ($p<0.01$) (Table 3).

Mean plant height across environments (Table 4) was 160.9 cm, and ranged from 115 to 186.4 cm. TZEI-12 × TZEI-13 recorded the lowest height (115 cm) while TZEI-39 × TZEI-22 recorded the highest height (186.4 cm). The result showed that there were highly significant differences among genotypes ($P<0.01$) for ear height (Table 3). Mean ear height (Table 4) was 76.9 cm ear height ranged from 52.1 cm (TZEI-12 × TZEI-13) to 97.2 cm (TZEI-35 × TZEI-19).

Meanwhile, TZEI35 × TZEI19 recorded the highest ear height while TZEI12 × TZEI 13 recorded the lowest ear height.

The result for days to silking showed that there were highly significant differences among genotypes ($P<0.01$). Days to silking ranged from 38 to 54 days. The mean days to silking (Table 4) was at 50 days. TZEI-45 × TZEI-47 was the latest to reach mid-silk, while TZEI-9 × TZEI-12 was the earliest to reach mid- silk.

From the result (Table 3), differences in days to tasseling among genotypes were highly significant ($p<0.01$). Mean days to tasseling (Table 4) was 47.6 days. Days to mid-tasseling ranged from 43 to 51.2 days. TZEI-12 × TZEI-13 recorded the highest number of days to tasseling while TZEI-9 × TZEI-12 recorded the lowest days to tasseling. The mean square (Table 3) for seed diameter showed highly significant differences among genotypes ($p<0.01$).

Seed diameter ranged from 0.7cm (TZEI-14 × TZEI-17) to 0.9 cm (TZEI-3 × TZEI-1). The result from the analysis for seed length (Table 3) indicated that differences among the genotypes were significant ($P<0.05$). Seed length ranged from 0.8 cm (TZEI-28 × TZEI-14) to 1.5 cm (TZEI-41 × TZEI-30) (Table 4). The data analysis for cob length (Table 3) revealed that differences among genotypes were highly significant ($P<0.01$). Mean cob length was 13. 4 cm and cob length ranged from 9.4 cm (TZEI-28 × TZEI-14) to 15.2 cm (TZEI-24 × TZEI-23) (Table 4).

The mean square analysis for cob diameter (Table 3) revealed that differences among genotypes were highly significant ($P<0.01$). Mean cob diameter (Table 4) was 4.2 cm. TZEI-42 × TZEI- 22 recorded the highest value (5.6 cm) while TZEI-28 × TZEI-14 recorded the lowest value (2.9 cm) for cob diameter.

Correlations among agronomic parameters measured

Identification of superior hybrids

The primary trait, grain yield, is a quantitatively inherited trait with low heritability. Several studies have indicated that highly significant phenotypic correlations between yield and many secondary traits can be found (Nzuve et al., 2014). The use of secondary traits in breeding can increase breeding progress as compared to selection for yield alone (Edmeades et al., 1997).

A superior maize hybrid must be high yielding and also must possess desirable agronomic characters. The correlation studies revealed that plant height was strongly associated with grain yield, ear height, days to tasseling, cob length and diameter, seed length and seed diameter. This indicates that any one of these traits could be used to select for the other.

The significant differences recorded for the different traits among the genotypes studied (Table 3) implied that the maize genotypes included in this study had diverse genetic backgrounds (Vashistha et al., 2013; Reddy et al., 2012).

Thus, the genetic variability recorded in this study could be exploited by plant breeders to develop hybrids adapted to the diverse environments in sub Saharan Africa to improve food security status (Feuillet et al., 2012). The significant genotype by environment interaction showed a wide variability with regard to the tested genotypes and the environments involved in this study (Alake et al., 2008).

Results on correlation among parameters are presented in Table 5. The correlation studies among traits showed that grain yield was positively correlated to days to tasseling, ear height, plant height, cob length, cob

Table 3. Mean squares values of combined ANOVA for agronomic traits of early maturing maize single cross hybrids evaluated during 2012 growing season.

Source of variation	Mean squares								
	DF	PHT	EHT	DS	DT	SDD	SDL	CL	CD
Replication	1	38.6	32.6	195.6	170	0.00794	0.00997	11.06	0.041
Environment	2	5878**	2297**	3135**	4253**	0.025*	0.0845**	147.95**	7.86*
Genotype	89	1156**	446**	19.07**	14.3**	0.0071**	0.01047*	6.318**	0.63**
Error	269	254	116	4.73	3.6	0.0042	0.0065	1.608	0.239
Total	539	-	-	-	-	-	-	-	-
Lsd	-	18.1	12.2	2.5	2.2	0.07	0.09	1.4	0.56
CV%	-	9.9	14	4.3	4	7.8	8.1	9.5	11.6

*= Significant (0.05%) **= Significant (0.001) ns= Non Significant Degree of Freedom = DF, Plant height = PHT, ear height = EHT, days to silking = DS, days to tasseling = DT, seed diameter = SDD, seed length = SDL, cob length = CL and cob diameter.

Table 4. Combined mean grain yield ($kgha^{-1}$) and agronomic performance of maize hybrids evaluated across three locations in Ghana during 2012 growing season.

Entry	Yield (kg)	Rank	DS	DT	PHT (cm)	EHT (cm)	CL (cm)	CD (cm)	SDL (cm)	SD (cm)
TZEI-36 × TZEI-39	6296	1	47.7	45.5	179	78	14.3	4.2	1	0.88
TZEI-33 × TZEI-19	6066	2	51.2	48.2	174	87	14	4.6	1.1	0.87
TZEI-35 × TZEI-19	5850	3	51.5	48.3	183	97	13.7	4.4	1.1	0.83
TZEI-45 × TZEI-34	5838	4	51	48.2	175	78	13.6	4.4	1	0.79
TZEI-22 × TZEI-45	5784	5	51	48.3	178	90	14.3	4.3	1	0.83
TZEI-2 × TZEI-22	5770	6	50.7	47.5	183	91	13.4	4.5	1.1	0.88
TZEI-34 × TZEI-7	5682	7	46.3	44.2	164	80	12.6	4.4	1	0.86
TZEI-10 × TZEI-11	5617	8	48	45.7	162	80	14.1	4.1	1	0.85
TZEI-48 × TZEI-45	5514	9	51.2	48.2	164	85	14.9	3.9	1	0.85
TZEI-22 × TZEI-48	5456	10	51	48.3	173	94	13.6	4.3	1	0.83
TZEI-34 × TZEI-46	5453	11	51.5	48.3	155	69	14.5	4.3	1	0.8
TZEI-11 × TZEI-15	5436	12	48.5	45.8	159	76	14.7	4.2	1	0.8
TZEI-41 × TZEI-30	5406	13	49.8	47.2	168	85	13.5	6	1	0.82
TZEI-25 × TZEI-23	5380	14	48	45.3	156	81	14	4.1	1	0.85
TZEI-48 × TZEI-20	5352	15	49.8	47.2	175	92	14.5	4.3	1	0.8
TZEI-12 × TZEI-15	5280	16	47.5	45.2	165	70	14.5	4.3	1	0.85
TZEI-3 × TZEI-1	5266	17	49.7	47.2	181	81	13.9	4.6	1	0.9
TZEI-41 × TZEI-47	5234	18	51	48.2	152	74	13.3	4	1.5	0.83
TZEI-31 × TZEI-7	5221	19	49.5	47	174	80	14.5	4.4	1.1	0.88
TZEI-36 × TZEI-35	5189	20	48	45.5	166	78	14.7	4.1	1.1	0.82
TZEI-30 × TZEI-47	5185	21	48	51	184	82	14.5	4.3	1	0.82
TZEI-42 × TZEI-30	5179	22	50.5	47.8	176	82	13.4	4.4	1	0.85
TZEI-27 × TZEI-19	5143	23	52.7	49.3	179	79	13.1	4.4	1.1	0.88
TZEI-13 × TZEI-10	5124	24	49.2	46.8	150	71	13.8	4.2	1	0.82
TZEI-25 × TZEI-14	5115	25	51.8	48.7	157	77	15	4.3	1	0.78
TZEI-38 × TZEI-36	5076	26	50	47.5	166	78	12.8	4.2	1	0.83
TZEI-1 × TZEI-19	5022	27	51.5	48.5	181	91	12.7	4.2	1	0.83
TZEI-22 × TZEI-46	5008	28	51	47.7	175	80	14.4	4.3	1	0.8
TZEI-17 × TZEI-15	4995	29	49	46.5	148	76	13.7	4.3	1	0.84
TZEI-13 × TZEI-17	4987	30	49.8	47	148	71	14.2	4.4	1	0.82
TZEI-14 × TZEI-17	4984	31	51.5	48.7	147	70	13.8	3.9	0.9	0.77
TZEI-35 × TZEI-16	4949	32	51	48.3	164	77	13.6	4.2	1	0.85
TZEI-48 × TZEI-16	4943	33	49.2	46.8	163	87	14.5	4	1	0.83
TZEI-12 × TZEI-20	4940	34	49.8	47.3	157	76	13.6	4.4	1	0.87

Table 4. Contd.

TZEI-19 × TZEI-48	4926	35	51.5	48.5	176	89	13.9	4.2	1	0.85
TZEI-46 × TZEI-34	4917	36	51.2	48.5	166	77	14	4.5	1.1	0.81
TZEI-39 × TZEI-36	4915	37	48	43.7	182	85	14.3	4	1	0.82
TZEI-14 × TZEI-15	4888	38	50.3	47.5	144	70	13.9	4.2	1	0.82
TZEI-36 × TZEI-34	4877	39	49	46.5	176	83	13.7	4.2	1.1	0.83
TZEI-36 × TZEI-33	4838	40	49.2	46.8	170	79	13.5	4.3	1	0.83
TZEI-11 × TZEI-9	4738	41	49.8	47.7	152	69	13.7	4.2	1	0.85
TZEI-12 × TZEI-9	4821	42	47.5	45	149	74	13.6	4.2	1	0.8
TZEI-24 × TZEI-12	4820	43	51.5	48.8	155	68	15.1	4.2	1	0.83
TZEI-4 × TZEI-3	4802	44	49.2	46.8	168	82	13.4	4.6	1	0.87
TZEI-36 × TZEI-38	4800	45	51.2	48.3	162	75	12.6	4.2	1	0.83
TZEI-2 × TZEI-19	4784	46	51.2	48.3	165	92	13.1	4.6	1	0.8
TZEI-22 × TZEI-18	4779	47	49.8	47.2	163	76	12.7	4.3	1	0.92
TZEI-27 × TZEI-14	4766	48	51.3	48.8	158	79	13.7	4.2	1	0.85
TZEI-25 × TZEI-27	4740	49	51.5	48.8	167	73	14.9	4.1	1.1	0.87
TZEI-19 × TZEI-46	4705	50	52.5	49.7	169	88	13.2	4.2	1.1	0.8
TZEI-9 × TZEI-12	4698	51	46	43.5	151	72	14.5	4	1	0.8
TZEI- 9 × TZEI-15	4676	52	46.3	44	142	68	12.8	4	1	0.87
TZEI-2 × TZEI-34	4675	53	50.2	47.5	154	74	13.1	4.5	1	0.81
TZEI-41 × TZEI-36	4642	54	48.7	47.2	159	71	13.2	4.1	1.1	0.8
TZEI- 33× TZEI-46	4631	55	52.7	49.7	166	80	13	4.2	1	0.85
TZEI- 33× TZEI-3	4587	56	50.7	47.7	165	79	11.9	4.8	1	0.82
TZEI-9 × TZEI-11	4558	57	52.3	49.3	147	69	11.7	4.1	1	0.87
TZEI-31 × TZEI-18	4521	58	49.7	46.7	167	77	13.7	4.2	1	0.87
TZEI- 10× TZEI-12	4509	59	49.5	46.8	149	71	13.6	4.1	1	0.8
TZEI- 18× TZEI-26	4505	60	50.5	47.3	166	83	12.4	4.2	1	0.83
TZEI9- × TZEI-10	4499	61	48.3	47.8	151	72	14.4	4	1	0.8
TZEI-42 × TZEI-47	4482	62	50.8	48.2	154	78	14.2	4.4	1.1	0.83
TZEI-22 × TZEI-20	4364	63	49.2	46.2	167	78	13.7	4	1	0.83
TZEI-47 × TZEI-34	4333	64	50.5	47.7	164	77	13.6	4.3	1	0.87
TZEI-36 × TZEI-22	4308	65	50.5	47.8	168	76	13	4.1	1	0.85
TZEI-34 × TZEI-3	4285	66	50	47	159	69	12.6	4.6	1.1	0.82
TZEI- 4 × TZEI-2	4261	67	51.5	48.7	161	73	12.2	4.6	1	0.85
TZEI-34 × TZEI-22	4228	68	51.2	48.5	165	77	12.1	4.3	1	0.82
TZEI33- × TZEI-2	4174	69	51.2	48	176	68	12.2	4.6	1	0.83
TZEI-22 × TZEI-20	4161	70	51.2	47.7	158	76	13.4	4.2	1	0.85
TZEI-39 × TZEI-22	4106	71	50.5	47.7	186	88	12.3	4.3	1	0.84
TZEI-39 × TZEI-34	4082	72	52.5	49.3	153	72	12.7	4.3	1	0.83
TZEI-14 × TZEI-16	4081	73	53.3	50.5	154	71	12.7	3.8	1	0.79
TZEI-30 × TZEI-31	4078	74	50.5	47.5	164	67	12.1	4.5	1.1	0.87
TZEI-41 × TZEI-22	4037	75	51.2	48.2	173	85	12.6	5.6	1	0.9
TZEI-24 × TZEI-23	4012	76	46.8	44.8	136	56	12.9	4.3	1	0.82
TZEI-39 × TZEI-30	3950	77	49.7	47.2	181	81	12.9	4.2	1	0.8
TZEI-19 × TZEI-18	3923	78	51.2	48.7	172	87	13.5	4.2	1	0.83
TZEI-27 × TZEI-9	3919	79	47.8	45.5	146	82	12	4	1	0.83
TZEI-23 × TZEI-15	3898	80	49.3	46.5	149	70	12.1	4.1	1	0.82
TZEI-18 × TZEI-46	3712	81	52.2	49	161	79	14.7	4.1	1.1	0.83
TZEI-36 × TZEI-20	3462	82	50.7	47.8	137	71	13.1	4.1	1	0.85
TZEI-38 × TZEI-35	3263	83	50.7	47.7	159	77	12.3	4.5	1	0.8
TZEI-17 × TZEI-16	2987	84	53.8	50.3	134	61	12.7	3.5	0.9	0.82
TZEI-32 × TZEI-5	2983	85	51.3	48.5	168	86	12.5	4.5	1	0.83
TZEI-11 × TZEI-12	2847	86	52.3	49.7	140	64	13.6	3.7	0.9	0.8

Table 4. Contd.

TZEI-28 × TZEI-14	2354	87	53.8	50.8	133	59	9.4	2.9	0.8	0.65
TZEI-46 × TZEI-47	1817	88	54.2	50.3	140	67	11	3.6	1	0.81
TZEI-12 × TZEI-13	1194	89	54.3	51.2	116	52	10.9	3.6	0.9	0.83
TZEI-45 × TZEI-47	1058	90	54.3	51	127	55	11.7	3.3	0.9	0.8
Grand mean	4598	-	50.3	48	161	77	13.6	4.2	1	0.83
CV (%)	20.7	-	4.3	4	9.9	14	9.5	11.6	8.1	7.8
LSD	1082	-	2.5	2.2	18.1	12.2	1.4	0.6	0.09	0.07

DS = 50 % days to silking, DT = 50 % days to tasseling, PHT = plant height, EHT = ear height, CL = cob length, CD = cob diameter, SDL = seed length, and SDD = seed diameter.

Table 5. Correlations among nine parameters of maize single cross hybrids evaluated across three locations in Ghana during 2012 growing season.

Variable	GY	DS	DT	EHT	PHT	SD	SDL	CL	CD
GY	1	-	-	-	-	-	-	-	-
DS	0.00328	1	-	-	-	-	-	-	-
DT	0.11558**	0.96858**	1	-	-	-	-	-	-
EHT	0.41004**	-0.36908**	-0.3321**	1	-	-	-	-	-
PHT	0.63331**	0.01782	0.10692*	0.66877**	1	-	-	-	-
SD	0.1900**	0.05539	0.07106*	0.11741**	0.22043**	1	-	-	-
SDL	0.36267**	0.03122	0.05746	0.25501**	0.33965**	0.47017**	1	-	-
CL	0.60951**	-0.04789	0.033	0.339**	0.45683**	0.24388**	0.39668**	1	-
CD	0.40185**	-0.00732	0.04979	0.30371**	0.39895**	0.31541**	0.38844**	0.3347**	1

** Highly significant (P<0.001), *significant (P<0.05), DT = days to 50% tasseling, DS = days to 50% silking, PHT = plant height, EHT = ear height, SD = seed diameter, SDL = seed length, CL = cob length, CD = cob diameter GY = grain yield.

diameter, seed length and seed diameter with the highest effect on plant height (r = 0.633) and cob length (r = 0.610) and ear height (r = 0.410).

The associations were highly significant (p < 0.001). This indicates that improvement on any of these characters could help improve grain yield. Similar results were reported by Bocanski et al. (2009) and Malik et al. (2005). They observed high and positive correlation between grain yield and plant height (r = 0.953), ear height (r = 0.867), and cob length (r = 0.959). Meanwhile, days to silking has no significant correlation with grain yield, plant height, seed length, seed diameter, cob length and cob diameter. This result is in agreement with Golam et al. (2011), who reported that grain yield and plant height did not correlate with days to silking. However, among agronomic traits, ear height, plant height, seed length, seed diameter, cob length and cob diameter were positively and significantly correlated, indicating that increase in any one of these traits could lead to increase in the other.

Ear height significantly correlated with plant height seed length, seed diameter, cob length, and cob diameter. The strong correlation between ear height and plant height with grain yield suggested that tall plants with high ear placement gave better yields compared to the shorter plants with lower ear placement. This could be attributed to the high dry matter accumulation function carried out by the high number of leaves possessed in the case of tall plants (Al-Tabbal et al., 2012).

There were negative correlations between ear height and days to silking and days to tasseling (r = - 0.369 and r = - 0.332). This result is in agreement with that of Malik et al. (2005). The negative correlation indicates that increase in days to silking and tasseling could indirectly reduce yield through stalk and root lodging (Malik et al., 2005). Plant height had low correlation with days to silking, but highly correlated with ear height (r = 0.668), days to tasseling (r = 0.10692), seed diameter (r = 0.2204), cob length (r = 0.4568), cob diameter (r = 0.3989) and seed length (r = 0.3396).

This result indicates that increase in plant height could lead to increase in these characters which could result in yield increase, since plant height has been observed to be controlled by the expression of many genes and the interactions between these genes (Bello et al., 2012). Therefore, traits that positively contribute the highest correlation with grain yield such as plant height, cob length, and ear height and cob diameter can be chosen as superior characteristics to help improve maize grain yield (Table 5).

Conclusion

The correlation studies among traits showed that grain yield was highly correlated with plant height, ear height, days to tasseling, cob length, cob diameter, seed length, and seed diameter with plant height contributing the highest effect (r = 0.633) followed by cob length (r = 0.610) and cob diameter (r = 0.402).This genetic diversity and the strong genetic association between grain yield and the agronomic traits would help in indirect selection thereby aiding breeders in the development of better hybrids for resource poor farmers.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Full Length Research Paper

Physico-chemical characterization of prickly pear (*Opunicia Ficus indica*) in the semi-arid region of Bahia State, Brazil

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In Brazil, prickly pear has gained increasing attention, mainly due to its nutritional benefits of health promotion, however, its postharvest conservation is still incipient and has to be evaluated. The objective of this work was to evaluate the physical and physico-chemical characteristics of prickly pear from three municipalities in the semi-arid region of the state of Bahia-Brazil. The fruits were harvested at random at the commercial harvest point stage, when the bark began to show a change of color from green to yellowish green. Data were subjected to analysis of variance, using the software GENES software, and the averages were compared using the Tukey test at the 5% probability level. The physical and chemical characteristics of the fruits were evaluated. Evaluating the effect of the place of cultivation on these characteristics, it is verified that the municipality of Guanambi presented the lowest value of acidity and higher pH, proving that there is climatic influence on the physico-chemical characteristics of the fruits. It was verified that the values found for the different variables studied, cactus pear, from the municipalities of Guanambi and Riacho de Santana present commercial properties more interesting for *in natura* consumption and agroindustrial use.

Key words: *Opuntia ficus, indica*, post-harvest quality, cultivation site.

INTRODUCTION

The cactus pear (*Opuntia ficus-indica*) is a plant of the cactus family, which supports conditions of lack of water, high temperatures, poor soils, and is easy to handle in planting. This is the reason why it is cultivated in the arid

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and semi-arid regions with the purpose of being used to feed livestock during periods of drought (Almeida et al., 2013).

In the northeast of Brazil, the cactus pear is mainly cultivated for feeding of animals. In the meantime, this cactus is the fruit known as the prickly pear, whose fruit is an oval berry with sweet and juicy pulp. It has high nutritional value, presenting in its composition fibers, soluble carbohydrates, vitamins (mainly A and C), calcium and magnesium (Yahia and Jacobo, 2011). In addition, it has the possibility of being explored for its medicinal properties, demonstrating several activities, including antioxidant (Zhong et al., 2010) and anti-inflammatory (Galati et al., 2003), and is a promising fruit with respect to the production of functional foods and nutraceuticals, because it has important compounds for humans. Based on this, prickly pear stands as one of the resources with great potential to add value and increase income and quality of life for the population of the Brazilian semi-arid region.

Although, the cactus pear is commonly cultivated for animal feed, its fruit is still little explored and valued, perhaps because it is considered as exotic fruit and with small spines. This fruit has not yet gained great space in the Brazilian market, due to the lack of knowledge on its potentialities (Almeida et al., 2013). Thus, the realization and dissemination of studies may serve for future research and stimulation of the production and consumption of still-consumed prickly pear in the country.

The physical and physicochemical characteristics of the fruits are influenced by soil and climatic conditions, cultural practices, harvesting period, genetic constitution, stage of maturation and postharvest treatment, among others (Nascimento et al., 2014). These characteristics are factors of quality and fundamental importance for subsidies related to the handling and packaging and for the elaboration of industrialized products (Chitarra and Chitarra, 2005). On the other hand, the physical characterization of the fruits has great importance with determination of the genetic variability of a species that can subsidize genetic breeding programs, as well as its relation with the environmental factors (Carvalho et al., 2003).

Thus, the objective of this work was to evaluate the physical and physicochemical characteristics of prickly pear from three municipalities in the semi-arid region of the state of Bahia.

MATERIALS AND METHODS

The fruits used in this work were *Opuntia fícus indica*, 3-year-old "Gigante" Cv, in properties of small farmers located in three municipalities in the semi-arid region of the state of Bahia, Guanambi, located in the Southwest region of Bahia, totally covered by the drought polygon, semi-arid climatic type, with geographic coordinates of south latitude 14° 13 '24 "and longitude 42° 46' 53" W, altitude 530 m; annual average temperature of 23.6°C and 720 mm annual average rainfall. Pindaí is located in the southwestern region of Bahia, with semi-arid climatic type, geographical coordinates with south latitude of 14° 29 '33 "and longitude 42° 41' 14" W, altitude 610 m. The average annual temperature in Pindaí is 23.1°C. The average annual rainfall is 688 mm. And Riacho de Santana, is located in the southwestern region of Bahia, semi-arid climatic type, with geographical coordinates: south latitude 13°36 '33 "and longitude: 42° 56' 20" W, altitude 627 m, with average temperature of 23.3°C and 852 mm of annual average rainfall.

The fruits were harvested in the month of March 2012, at random, at the commercial harvest point stage, when the bark began to show color change, from green to yellowish green. After being harvested, they were taken to the Food Analysis Laboratory of the Federal Institute of Bahia, Guanambi Campus for physical and chemical analysis. In the laboratory, the spines were removed with the aid of a soft sponge, washed and selected, those that presented some types of injury were discarded. Afterwards they were separated into 10 lots of 5 fruits each, totaling 50 fruits per municipality.

Separation of the bark, pulp and seeds was performed manually using knife, spoon and fine mesh stainless steel sieve. The fruits were submitted for physical determinations: length, diameter, flesh firmness, peel color, bark thickness, number of seeds, fruit weight, bark weight, pulp weight and pulp/bark ratio. Measurements of longitudinal length and diameter (cm), was determined by means of direct measurements, with the aid of a caliper, placing it in a perpendicular position and parallel to the axes of the fruit; the mass (g) was determined by individual weighing of each fruit on a digital analytical scale; firmness of the whole fruit with bark, was determined individually in two distinct points of the equatorial region in the whole fruit with digital dynamometer (N), the coloration was realized by means of a Color Flex 45/0 (2200) colorimeter, stdzMode: 45/0 with reading direct reflectance of the coordinates L* (brightness) a* (red or green tint) and b* (yellow or blue tint) of the Hunterlab Universal Software system. From the values of L*, a* and b*, the angle hue (°h*), number of seeds by individual counting in each fruit and the fruit pulp/peel ratio were calculated by direct weighing in an analytical balance for the average evaluation of fruit weight, as well as the proportions of pulp and peel.

The pulp was analyzed for the physico-chemical characteristics: pH, titratable acidity, soluble solids and SS/AT ratio following the methodologies described in the Adolfo Lutz Institute's Manual of Analyzes (IAL, 2008). The pH was was measured by direct measurement in potentiometer of bench, with glass membrane electrode calibrated with pH 4.0 and 7.0 solutions; titratable acidity was determined by titration with 0.1 N sodium hydroxide using 1% phenolphthalein as indicator, and the results were expressed as % citric acid; soluble solids was determined by direct reading in digital refractometer and the results expressed in °Brix, the SS/AT ratio is the soluble solids value divided by the titratable acidity and the result expressed in pure number with two decimal places.

The experiment was conducted using a completely randomized design, with ten replicates and five fruits per experimental unit, three treatments (municipalities). The data were subjected to analysis of variance using the software GENES software, and the averages were compared using the Tukey test at the 5% probability level (Cruz, 2001).

RESULTS AND DISCUSSION

The values of the physical and physico-chemical components of the fruits from the municipalities of Guanambi, Pindaí and Riacho de Santana are presented

Table 1. Values of the physicochemical analyses of prickly pear from the municipalities of Guanambi, Pindái and Riacho de Santana.

Characteristic	Municipality			Medium	CV (%)
	Guanambi	Pindaí	Riacho de Santana		
Length (cm)	9.38[a]	7.65[c]	8.9[b]	8.64	7.5
Diameter (cm)	5.73[a]	5.83[a]	5.86[a]	5.81	7.7
Fruit weight (g)	180.68[b]	143.65[c]	190.43[a]	171.59	11.5
Pulp weight (g)	106.42[a]	72.62[c]	96.21[b]	91.75	15.4
Shell weight (g)	72.52[b]	68.74[b]	86.25[a]	75.83	16.3
Pulp/shell ratio	1.55[a]	1.08[b]	1.12[b]	1.25	29.1
Number of seeds	308.12[a]	277.72[c]	288.24[b]	291.36	6.3
Shell thickness (cm)	0.50[b]	0.55[ab]	0.57[a]	0.546	21.5
Firmness (N)	27.29[a]	27.13[ab]	26.78[b]	27.06	3.02
Angle HUE°	60.28[c]	80.18[a]	79.16[b]	73.21	2.3
Soluble solids	14.19[a]	7.59[c]	13.33[b]	11.70	6.2
pH	6.02[a]	5.65[b]	5.08[c]	5.58	6.3
Titratable acidity	0.241[b]	0.153[c]	1.12[a]	0.507	13.3
Relation SS/AT	59.19[a]	57.83[a]	11.90[b]	42.97	30.5

*Averages followed by the same letter in the rows do not differ from each other by Tukey test at 5% probability.

in Table 1. According to data analysis, it was observed that there was a significant difference in fruit length in the three municipalities evaluated. The fruits from Guanambi presented a 9.38 cm length significantly higher than the values obtained in prickly pear of the other localities: 7.65 (Pindaí) and 8.9 cm (Riacho de Santana). Similar results are demonstrated by Almeida et al. (2013) (9.24 cm) and Silva Junior et al. (2007) (9.18 cm). As for the diameter characteristic, there was no significant difference between the evaluated municipalities, presenting an average of 5.81 cm, this is within the values obtained by Silva Junior et al. (2007) from 4.2 to 5.9 cm, similar to those found by Almeida et al. (2013) (5.73 cm) and higher than that found by Oliveira et al. (2011) (5.1 cm) and Chougui et al. (2013) (4.45 cm).

The fresh fruit weight varied from 143.65 to 190.43 g, the municipality of Riacho de Santana presented the highest fresh fruit weight of 190.43 g, followed by the municipalities of Guanambi (180.68 g) and Pindaí (143.65 g). It is known that several factors can influence the average weight of the fruits, such as irrigation, fertilization, harvesting time, stage of maturation, cultural treatments and climate (Nascimento et al., 2014). This work shows that the region of production had a significant influence on the average weight of the fruits, since the municipalities with the highest annual rainfall presented fruits with higher average weights. As for pulp and bark mass, the municipality of Riacho de Santana also presented higher values than the other municipalities (96.21 g and 86.25, respectively). It is observed that prickly pear have a large amount of pulp when compared with other cactus, and this characteristic may be interesting both for the *in natura* consumption and for processing of the fruit.

The average values found in this work for fresh fruit, pulp and peel (171.59, 91.75 and 75.83 g) were higher than those reported by Almeida et al. (2013) (154.62, 66.49 and 66.19 g, respectively). Such divergences are probably due to differences in the soil-climatic conditions of the growing sites. Brunini et al. (2004) also observed differences in acerola from different growing regions.

The fruit pulp/peel ratio ranged from 1.12 to 1.55, and the highest values were found in fruits from Guanambi. These values are similar to those reported by Rojas et al. (2008) and Chiacchio et al. (2006). Regarding the quantity of seeds, the importance of this component is related to the size of the fruit and therefore, related to the yield and also quality of the product. The number of seeds ranged from 277.72 to 308.12, the fruits from Guanambi had the highest amount of seeds. These values are higher than those found by Almeida (2011) (257).

For thickness of the bark, the values ranged from 0.50 to 0.57 cm, where the fruits from Guanambi presented the lowest values (0.50 cm) and Riana de Santana had 0.57 cm, while the fruits of Pindaí presented intermediate values of 0.55 cm. The thickness of the shell is of great importance in the yield, because the smaller the thickness, the greater the pulp yield of the product. Prickly pear showed firmness values of 26.78, 27.13 and 27.29 N for the municipalities of Riacho de Santana, Pindaí and Guanambi, respectively. The fruits from Guanambi were firmer, proving to be more resistant to the mechanical damages. These results are consistent with those found by Zegbe and Covarrubias (2010) (27.3).

The color of the bark, evaluated through the hue angle, presented significant differences among the evaluated municipalities. The fruits from Pindaí and Riacho de Santana had a yellow coloration of 79.16 and 80.18, while fruits harvested in Guanambi showed a reddish orange coloration of 60.28. These differences were probably due to the effect of different environmental conditions. Brito Primo (2008) also observed color differences when evaluating fruits of *Opuntia fícus* indica cv Gigante in different locations in the state of Paraíba.

The soluble solids contents varied between the different localities, ranging from 7.59 to 14.19 °Brix. Fruits harvested in Guanambi present the highest soluble solids contents, differing from other localities. It is shown to be more attractive for *in natura* consumption, because they present a higher content of soluble solids and thus a better palatability. Similar results were found by Brito Primo (2008), evaluating fruits in different locations in the state of Paraíba, and by Chougui et al. (2013), which found values of 12 to 15 °brix in varieties grown in northern Algeria.

The pH and acidity values can be observed to vary from 5.08 to 6.02 and 0.153 to 1.12, respectively. These values make the prickly pear flavored moderately and are well accepted for consumption as fresh fruit since, together with a high soluble solid content, it gives the product a bittersweet flavor. The values of pH and acidity found in this work are consistent with those found by Zegbe and Covarrubias (2010). However, Chougui et al. (2013) verified that prickly pear have high pH (6.2-6.6) and low acidity (0.04 to 0.07%). Almeida et al. (2013), also found low acidity for prickly pear of 0.08 in fruits grown in the state of Bahia.

Evaluating the effect of the place of cultivation on these characteristics, it was verified that the municipality of Guanambi presented the lowest value of acidity and higher pH, proving that there is climatic influence on the physico-chemical characteristics of the fruits. Nascimento et al. (2014) evaluating the physical and physico-chemical characteristics of mangabeira fruits also verified significant differences for the values of pH and acidity in the different cultivation sites.

The SS/AT ratio ranged from 11.90 to 59.19, and fruits from the municipalities of Guanambi and Pindaí presented the highest values for the SS/AT ratio (59.19 and 57.83, respectively), while fruits in the municipality of Riacho de Santana presented the lowest values. The SS/AT ratio is widely used as a subjective taste assessment criterion because it expresses the sweet-acid balance of foods. It is interesting that this relation presents high values, since it indicates there are more sugars than fruit acids. Thus, the fruits from the municipality of Riacho de Santana contain more acids than sugars.

Conclusion

The physical and physico-chemical characteristics of prickly pear are strongly influenced by the growing site, especially in relation to color, fruit mass and soluble solids contents. Factors such as genetic variability, edaphoclimatic conditions and crop management may be determinants of the oscillations recorded for the variables studied in the different orchards.

Considering the values found for the different variables studied, prickly pear from the municipalities of Guanambi and Riacho de Santana present commercial properties more fascilitating for *in natura* consumption and agroindustrial use.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Full Length Research Paper

Comparison of GGE biplot and AMMI analysis of multi-environment trial (MET) data to assess adaptability and stability of rice genotypes

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Genotype × Environment (G×E) interaction and stability performance were investigated on paddy yield of eighteen rice genotypes and twelve locations using two well renowned statistical models; genotype main effect and G×E Biplot analysis (GGE) and additive main effects and multiplicative interaction (AMMI) analysis. The aim of this study was to elucidate the performance of some advance rice lines/genotypes at multiple locations in multi environment trials (METs) using GGE biplot and AMMI analyses. The results of GGE biplot and AMMI analyses performed over the data of paddy yield at multiple locations of two years 2014 and 2015 indicated that G×E interaction plays a crucial role in determining the performance of genetic material in METs. The results declare that GGE and AMMI not only provide easy and affective evaluation of genotypes into environment interactions in a number of locations but also a comprehensive understanding of the variability of the target locations. AMMI analyses for data of both years indicated that RRI 7 was the highest priority selected genotype for six locations, NIAB 1175 for four and RRI 3 for three locations. Dhokri and Kala Shah Kaku were the highest yielding, while Faisalabad and Dhokri were the most stable environments in 2014. Likewise, Faisalabad and PARC Islamabad were the highest yielding as well as most stable environments in 2015. Basmati 515 and PS 2 were the most favorable genotypes in 2014 and 2015, respectively for their high paddy yield and stability at all locations. The results further suggested that both models were useful and presented similar interpretations about MET data.

Key words: Genotype main effect and G × E biplot analysis (GGE), additive main effects and multiplicative interaction (AMMI) analysis, rice, fine type, multiple locations.

INTRODUCTION

Rice is being used as staple food by more than three billion of world population which represents 50 to 80% of their daily calorie intake (Khush, 2005; Amirjani, 2011). This population will increase to over 4.6 billion by 2050 (Honarnejad et al., 2000) which will demand more than 50% of rice needs to be produced to cope with the growing population (Ashikari et al., 2005; Srividya et al., 2010). Rice is the second most important staple food

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crop in Pakistan not only in local consumption but also in view of large exports (Anonymous, 2006). In Pakistan, rice was grown on approximately 2.89 million hectares with a total production of 7.01 million tonnes and earned a foreign exchange of worth US$ 1.53 billion. It accounts for 3.2% in value added in agriculture and 0.7% of Gross Domestic Product (Pakistan Economic Survey, 2015).

Rice production in Pakistan is confined in four, more or less distinct agro-ecological zones. Each zone represents diverse edaphic, hydrological and climatic conditions. Genotypes with wide adaptability, which can perform consistently well over a range of environments is required. Crop stability, is the ability of a crop to exhibit minimum interaction with both predictable and unpredictable environments (Qayyum et al., 2000). Rice yield in Pakistan is very low as compared to other rice growing countries of the world. With the passage of time, due to drastic climatic changes, there are developing vast differences in agro-climatic conditions among different locations of Pakistan. These phenomena make new developing rice varieties more unstable when grown under diverse environmental conditions and results in poor yield (Duncan, 1955).

Genotype × Environment (G × E) interaction is important to plant breeder for examining yield stability of genotypes (Davis and Rutgar, 1976). Linnemann et al. (1995) stated that it is important to understand crop development in relation to biophysical conditions and changes in season when selecting well-adapted genotypes and correct planting date. Varieties that show low G × E interaction and have high stable yields are desirable for crop breeders and farmers, because due to their genetic composition their yields are larger that indicates that the environments have less effect on the performance of genotypes. Therefore, evolution of rice varieties that have high yield and stability in performance over a wide range of environments will remain an important criterion in rice breeding (Tai, 1971).

To achieve this goal, innovative statistical techniques like Genotype main effect and G × E biplot analysis (GGE) and additive main effects and multiplicative interaction (AMMI) analysis will be needed to compare the lines and testing environments in order to separate rice genotypes with high adaption capability to diverse environments for accomplishing specific socio-economic and agricultural needs (Prasad and Singh, 1990; McLaren and Chaudhary, 1994; Kueneman, 2006; Khush et al., 1979). The objective of the present study is to present the idea how to interpret results of GGE biplot and AMMI analyses to study G × E interactions and stability in performance of advance fine rice genotypes grown at different locations of Pakistan.

MATERIALS AND METHODS

Studies were conducted under the agro climatic conditions of experimental farm of research area at nine different locations, viz., Dhokri, Dera Ismael Khan, Faisalabad, Gujranwala, for assessing the performance of twelve Basmati rice lines, that is, BR-1, BR-18, BR-23, DR-65, NIAB-1114, NIAB-1175, PK 8431-1-2-1-2-4, PK 8660-13-3-1, PK 8685-5-1-1-1-1, PK BB-4, Rachna-1080, and RRI-7 including two check varieties, that is, Basmati 515 and Super Basmati in 2014. While in the second year 2015, NIAB-1114, NIAB-1175, PK BB-1, PK BB-4, PK 9408-8-1-2-2, RRI-3, and RRI-7 were used including one check variety, that is, PS 2, to be evaluated in Regional Adaptability Yield Trials.

One month old seedlings were transplanted at plant to plant and row to row distance of 20 cm. The experiments were sown in randomized complete block design (RCBD) in a triplicate fashion at each site. To avoid the chance of mortality, two to three seedlings per hill were placed. The fertilizer was applied at the rate of 120 N + 60 P_2O_5 + 0 K_2O kg/ha, whereas in aromatic group at the rate of 80 N + 40 P_2O_5 + 0 K_2O kg/ha. During crop growth stages, standard agronomic practices were followed. The quality characters such as size (length, length/width, length/width × thickness) were carried out according (Kush et al., 1979; Sagar et al., 1988; Kawai and Amano, 1991; Singh, 1997). Paddy yield was recorded after the harvest of the crop. At maturity, yield was recorded and subjected to analysis of variance (ANOVA) as suggested by Steel and Torrie (1980) to determine the significance of genotypes, environments and genotype environment interactions.

GGE biplot is being rigorously used for analyzing multi-environment trail (MET) data, which provides a working guide for breeders on biplot analysis and interpretation of results (Domitruk et al., 2001). Earlier, many studies have been interpreted using simple statistical techniques. Therefore, advance statistical techniques were used for this MET data in order to interpret three important results necessarily required in multi-location trials such as identifying high yielding and most stable genotype, best test environment and best performing genotype in specific environment. These questions become very hard in such METs that involve a large number of genotypes.

RESULTS AND DISCUSSION

GGE biplot analysis

Biplots graphs (Figure 1a and b) are plotted where aspects of all the Basmati rice genotypes and environments are on the same axes so that the interrelationship can be easily depicted. Therefore, biplot 1a explains 65.64% of the total variations while Figure 1b explains 77.61% of the total variations. In both biplots, all the genotype and environment vectors are drawn showing the specific interactions between genotypes and environments.

Based on Figure 1a and b, it is possible to assess both mean yields and stability performance through biplot environments with shorter/acute angle between their vectors are more correlated while those with larger/obtuse angle are negatively correlated. Shorter the angle, higher the value of positive correlation between two locations. Presence of close associations among the environments suggests that the same information about the behavior of studied genotypes was obtained from other locations and performance of all the genotypes was same at these locations. The location vectors are lines that connect the biplot origin and the markers of test locations and the angle between them is related to the

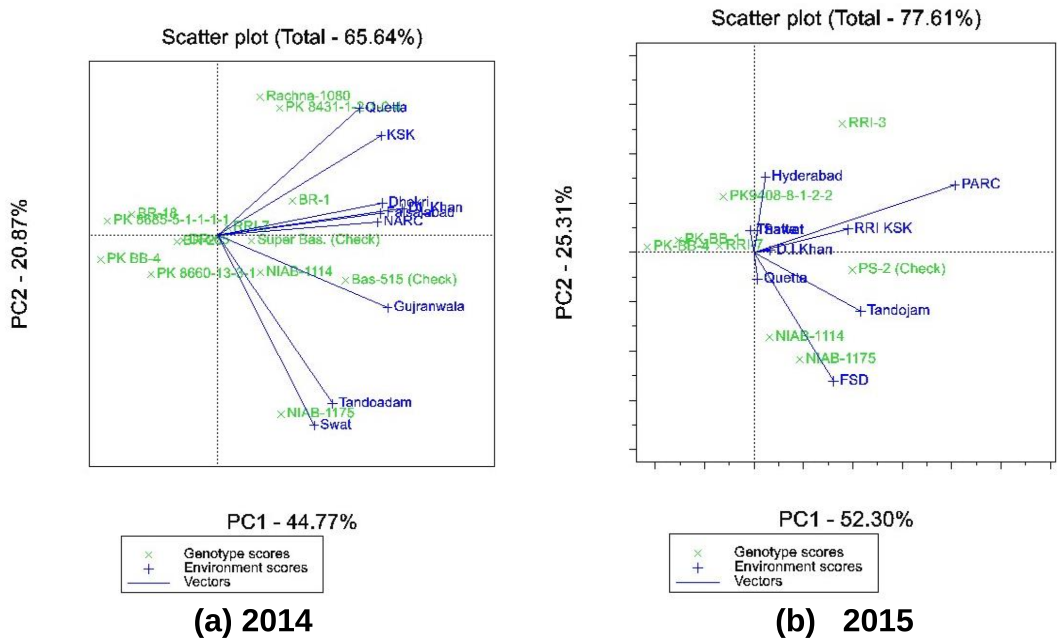


Figure 1. Vector view of GGE biplot for relationships among locations/environments during 2014 and 2015.

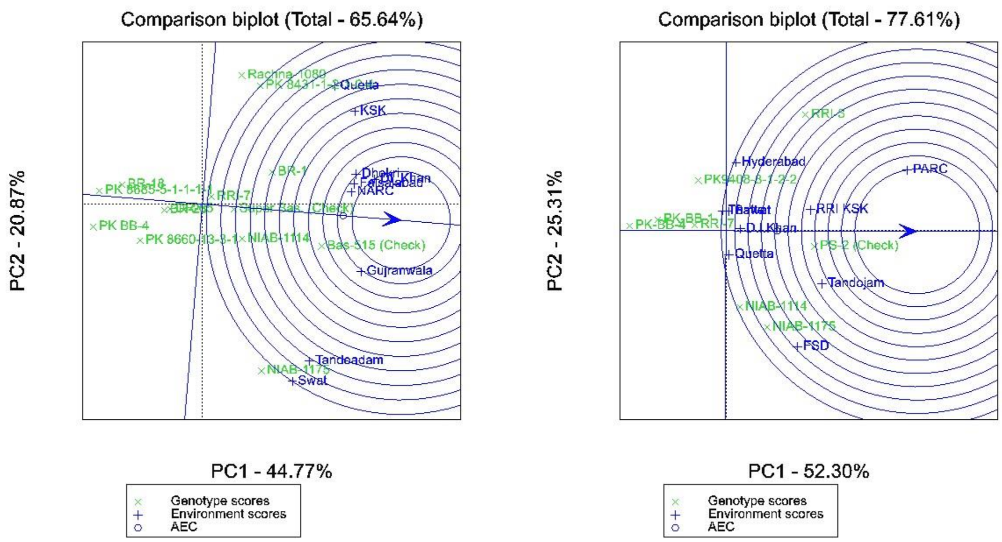
correlation coefficient. Therefore, the results suggest to drop one of these locations in order to secure resources and any other location may be added having different environmental conditions. For example, in the year 2014, locations Dhokri, Faisalabad, D.I. Khan and NARC Islamabad have shorter angles and thus show higher correlation among themselves. Therefore, it will be wiser to consider any one of these locations next year that will be representative of all these locations in order to secure inputs.

GGE biplot can also be used to interpret genotypes with similar response to the target environments. Two genotypes can be easily compared by joining them with straight lines passing through them and biplot origin. Based on Figure 1a and b, it can be visualized that genotypes that have shorter angle or are in close proximities on biplots indicate their similar response to the environments. For example, Rachna-1080 and PK 8431-1-2-1-2-4 showed similar response to environments tested in 2014, while response of NIAB-1175 was very different in comparison with these two. NIAB-1175 and NIAB-1114 have shorter or acute angle between them indicating that these two responded similarly and the differences between them was proportional in all target locations. Genotypes located at more distance from center of biplot are less stable and near to origin genotypes are more stable (Jones, 1926). RRI-7 are more stable than NIAB-1114 in 2015.

An average tester coordinate (ATC) horizontal and vertical axes is drawn such that it passes through the biplot origin and the average location and show the stability and mean yield of each genotype (Yan and Nicholas, 2006). Genotypes and environments on the same parallel line or ordinate have similar yield and a genotype or environment on the right side of the mid-point of ATC vertical axis has higher yield than those of left hand side. Figure 2a and b shows that PK BB-4 was the least yielding genotype among all the studied locations as the angle of its vector is obtuse with respect to all the environment vectors, in both years. While the check variety Basmati 515 was above average in 2014 and PS 2 check variety in 2015, in all the locations as indicated by the longest vector and acute angle with respect to all the other location vectors.

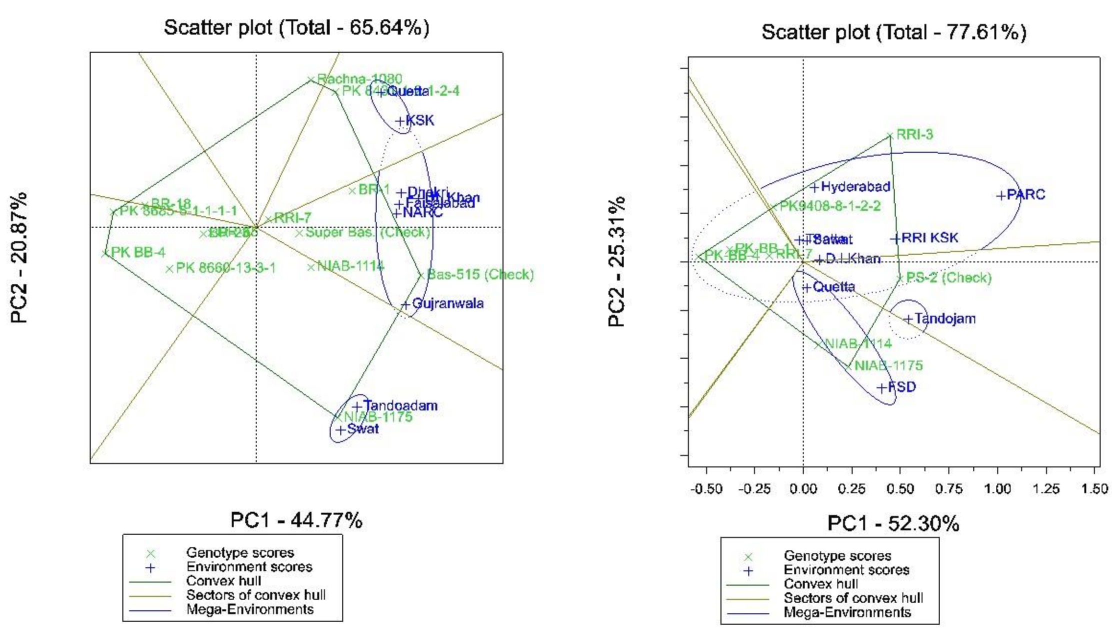
The smaller the absolute length of projection of a genotype, the more stable it is (Fehr, 1987). The biplots in Figure 2a and b further showed that NIAB 1147, Rachna 1080 and PK 8431-1-2-1-2-4 were the least stable genotypes and showed unstable yield performance at all the locations in 2014 as indicated by longer vectors/distance from ATC horizontal. During 2015, NIAB 1175, NIAB 1114 and RRI 3 were the least stable genotypes, as indicated by the longer distances from ATC horizontal. Super Basmati and RRI 7 were the most stable genotypes in 2014, while RRI 7, PK BB-4 and then PS 2 were the most stable in year 2015 as indicated by shorter distances from ATC horizontal axis (Figure 2a and b).

According to the biplot shown in Figure 3a and b, the corner genotypes that are the most responsive ones, can be visually determined. These corner genotypes were PK 8685-5-1-1-1-1, Rachna 1080, Basmati 515, NIAB 1175 and PK BB 4 in 2014 whereas PK BB 4, RRI 3, PS 2 and NIAB 1175 during the next year, 2015. Further, locations



(a) 2014 **(b) 2015**

Figure 2. GGE biplot of ideal genotype and comparison of the genotypes with the ideal genotype (center of the concentric circles) for year 2014 and 2015.



(a) 2014 **(b) 2015**

Figure 3. GGE biplot identification of winning genotypes and their related mega-environments for year 2014 and 2015.

are divided into three sectors or mega-environments in both years. During the first year, 2014, the first sector represents Quetta and Kala Shah Kaku (KSK) with genotype Rachna and PK 8431-1-2-1-2-4 as the most favorable genotypes. The second sector represents Dhokri, D.I.Khan, NARC Islamabad, Faisalabad, Dhokri and Gujranwala, with genotype Basmati 515 as the most favorable. The third sector represents Tondojam and

Table 1. AMMI analysis showing genotype means and scores for years 2014 and 2015.

Genotype	Mean yield (t/ha)	IPCAg[1]	IPCAg[2]
(a) 2014			
Bas-515 (Check)	3.593	16.09175	-4.67193
BR-1	3.526	13.36208	3.71975
BR-18	3.184	6.75859	14.33930
BR-23	3.498	6.88055	13.67038
DR-65	2.929	17.26309	19.30991
NIAB-1114	4.036	-41.06903	-6.34334
NIAB-1175	4.229	-12.91891	-27.36193
PK 8431-1-2-1-2-4	3.712	-41.00750	2.57288
PK 8660-13-3-1	3.618	23.90716	-13.43998
PK 8685-5-1-1-1-1	3.850	4.18955	-35.37994
PK BB-4	3.570	0.78376	6.59637
Rachna-1080	3.712	16.28548	-6.37743
RRI-7	3.758	-23.35728	23.96435
Super Bas. (Check)	3.542	12.83071	9.40160
(b) 2015			
NIAB-1114	3.312	-2.86682	-20.35945
NIAB-1175	3.377	-13.31683	-25.82723
PK-BB-1	2.988	17.94437	3.20326
PK-BB-4	2.845	23.66649	1.55312
PK9408-8-1-2-2	3.089	4.92062	13.44066
PS-2 (Check)	3.632	-23.93160	-4.42479
RRI-3	3.556	-22.82621	30.59649
RRI-7	3.257	16.40997	1.81793

Swat, with genotype NIAB 1175 as the most favorable. Likewise, during second year 2015, first sector represents Hyderabad, PARC Islamabad, Swat, RRI Kala Shah Kaku, D.I. Khan, with genotype RRI 3 as the most favorable genotypes. The second sector represents Quetta and Faisalabad, with genotype NIAB 1175 as the most favorable. The third sector represents Tondojam, with genotype PS 2 as the most favorable. Also, those genotypes within the polygon were less responsive to location than the corner genotypes. For example, in the second sector of locations in 2014, NIAB 1114 and Super Basmati were less responsive to corner winning genotype, that is, Basmati 515 at this specific location.

AMMI analysis

The mean paddy yield (t/ha) value (Table 1a and b) of genotypes averaged over environments indicated that the genotype NIAB 1175 has the highest (4.2 t/ha) paddy yield followed by NIAB 1114 (4.03 t/ha), PK 8685-5-1-1-1-1 (3.85 t/ha) and RRI-7 (3.75 t/ha) during the first year while during the second year PS 2 showed the highest (3.63 t/ha) paddy yield followed by RRI-3 (3.55 t/ha) and NIAB 1175 (3.37 t/ha). The environments mean paddy yield ranged from Dhokri (4.88 t/ha) to Quetta (2.41 t/ha) in 2014, whereas Faisalabad (4.64 t/ha) to DI Khan (0.33 t/ha) during the second year 2015. Table 2a and b shows IPCA[1] and IPCA[2] scores, which represent the adaptability of a genotype over environments and association between genotypes and environment (Albert, 2004). Regardless of the positive of negative signs, genotypes with large scores have high interactions and unstable, whereas genotypes with small scores or close to zero have low interaction and stable (Zobel et al., 1988). Therefore, RRI Kala Shah Kaku and Hyderabad were the most stable and rich environments while Swat and PARC Islamabad are the most unstable and poor environments among all in 2014 and 2015, respectively. The same results are shown in GGE biplot indicated by the length of the vectors.

Based on IPCA[1] scores and multiplicative interaction between IPCAs of genotypes and environment, ranking of the genotypes in each environment can be visualized. Table 3a and b shows the ranking of the genotypes according to their performances in respective environments for the year 2014 and 2015, respectively. The candidate variety RRI-7 performed best in two locations (Dhokri and Faisalabad) in terms of yield and four locations (Thatta, Swat, Quetta and DI Khan) in 2014

Table 2. AMMI analysis showing environment (Location) means and scores for years 2014 and 2015.

District	Mean (t/ha)	Variance (margin = 1236)	IPCAe[1]	IPCAe[2]
(a) 2014				
Dhokri	4.886	119	5.47188	22.63350
DI_Khan	3.079	489	27.12555	12.51417
Faisalabad	3.176	255	4.75110	10.22964
Gujranwala	3.908	139	8.71778	6.49590
KSK	4.659	1148	-3.76996	-45.14122
NARC	2.824	785	-17.25875	-21.22751
Quetta	2.412	125	11.77937	10.90798
Swat	3.920	2388	-62.77031	14.95285
Tandoadam	3.764	555	25.95335	-11.36531
(b) 2015		**Variance (margin =2489)**		
D.I.Khan	0.325	16	9.24895	0.70044
FSD	4.637	541	-5.84538	-33.79006
Hyderabad	4.454	262	9.09141	19.96271
PARC	4.592	1006	-36.86304	17.24029
Quetta	3.668	36	13.70134	-6.71723
RRI KSK	3.369	424	-7.48116	6.08910
Swat	0.934	97	14.83913	5.95706
Tandojam	3.608	365	-12.91098	-15.52964
Thatta	3.725	66	16.21973	6.08733

Table 3. First four AMMI selections per environment for years 2014 and 2015.

Environment	Mean (t/ha)	Score	1	2	3	4
(a) 2014						
DI Khan	3.079	27.13	PK 8660-13-3-1	Rachna-1080	Super Bas. (Check)	Bas-515 (Check)
Tandojam	3.764	25.95	PK 8660-13-3-1	PK 8685-5-1-1-1-1	Rachna-1080	NIAB-1175
Quetta	2.412	11.78	Rachna-1080	Super Bas. (Check)	NIAB-1175	PK 8660-13-3-1
Gujranwala	3.908	8.72	NIAB-1175	Rachna-1080	PK 8660-13-3-1	Super Bas. (Check)
Dhokri	4.886	5.47	RRI-7	BR-23	Super Bas. (Check)	PK BB-4
Faisalabad	3.176	4.75	RRI-7	NIAB-1175	NIAB-1114	Rachna-1080
KSK	4.659	-3.77	NIAB-1175	PK 8685-5-1-1-1-1	NIAB-1114	PK 8660-13-3-1
NARC	2.824	-17.26	NIAB-1175	NIAB-1114	PK 8685-5-1-1-1-1	PK 8431-1-2-1-2-4
Swat	3.920	-62.77	NIAB-1114	PK 8431-1-2-1-2-4	RRI-7	NIAB-1175
(b) 2015						
Thatta	3.725	16.22	RRI-7	RRI-3	PK-BB-1	PK9408-8-1-2-2
Swat	0.934	14.84	RRI-7	RRI-3	PK-BB-1	PS-2 (Check)
Quetta	3.668	13.70	RRI-7	NIAB-1114	NIAB-1175	PS-2 (Check)
D.I.Khan	0.325	9.25	RRI-7	PS-2 (Check)	RRI-3	NIAB-1114
Hyderabad	4.454	9.09	RRI-3	RRI-7	PK9408-8-1-2-2	PS-2 (Check)
Faisalabad	4.637	-5.85	NIAB-1175	NIAB-1114	PS-2 (Check)	RRI-7
RRI KSK	3.369	-7.48	RRI-3	PS-2 (Check)	NIAB-1175	NIAB-1114
Tandojam	3.608	-12.91	PS-2 (Check)	NIAB-1175	NIAB-1114	RRI-3
PARC	4.592	-36.86	RRI-3	PS-2 (Check)	NIAB-1175	PK9408-8-1-2-2

and 2015, respectively. Likewise, genotype NIAB-1175 performed best in three locations (Gujranwala, Kala Shah Kaku and NARC) and one location (Faisalabad) in 2014 and 2015, respectively.

Conclusion

Genotype NIAB 1175 has the highest (4.2 t/ha) paddy yield followed by NIAB 1114 (4.03 t/ha), PK 8685-5-1-1-1-1 (3.85 t/ha) and RRI-7 (3.75 t/ha) in the first year while in the second year, PS 2 showed the highest (3.63 t/ha) paddy yield followed by RRI-3 (3.55 t/ha) and NIAB 1175 (3.37 t/ha). The environments mean paddy yield ranged from Dhokri (4.88 t/ha) to Quetta (2.41 t/ha) in 2014, whereas Faisalabad (4.64 t/ha) to DI Khan (0.33 t/ha) in the second year 2015.

PK BB-4 was the least yielding genotype among all the studied locations in both the years. Super Basmati and RRI 7 were the most stable genotypes in 2014, while RRI 7, PK BB-4 and then PS 2 were the most stable in year 2015. While the check variety Basmati 515 was above average in 2014 and PS 2 check variety in 2015, in all the locations. NIAB 1147, Rachna 1080 and PK 8431-1-2-1-2-4 were the least stable in 2014, while NIAB 1175, NIAB 1114 and RRI 3 were the least stable genotypes in 2015. RRI Kala Shah Kaku and Hyderabad were the most stable and rich environments, while Swat and PARC Islamabad are the most unstable and poor environments among all in 2014 and 2015, respectively.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Full Length Research Paper

Determinants of smallholder farmers' awareness of agricultural extension devolution in Kenya

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The devolved governance structure in Kenya places the provision of agricultural sector services such as extension at the local units. However, farmers' awareness on this aspect and where services are available remains limited and often, there is confusion. In order to provide clarity and enhance the understanding of extension service delivery, this study sought to characterize farmers' awareness of agricultural extension devolution and analyze factors that influence their awareness. Data was collected in Meru County using semi-structured questionnaires through face-to-face interviews on a representative multi-stage sample of 288 farmers. A binary logit model was applied to analyze the determinants of farmers' awareness. Slightly less than half of the respondents indicated that they were aware of agricultural extension devolution. The factors that were found to significantly enhance awareness are attendance to farmer field days, land tenure security, income and education.

Key words: Extension-devolution, farmers, awareness, Kenya.

INTRODUCTION

In most agricultural policy debates, a consensus exists that agricultural extension is a key component in enhancing agricultural productivity and profitability. The term 'agricultural extension' is contextualized here to mean the whole arrangement of organizations that facilitate agricultural stakeholders to obtain relevant information, skills and technologies to improve the livelihoods of farmers and others who depend on farming. In Kenya, agricultural extension dates back to the early 1900s and has undergone various reforms since then. The integrated policy approach of 1960s achieved remarkable success in the dissemination of hybrid maize technology although the policy suffered from ineffective management, poor co-ordination and lack of community engagement. The training and visit system of agricultural extension that was implemented mainly in the 1980s to early 1990 succeeded in improving staff quality through training and the establishment of better extension linkages but there was no evidence of sustainable impact on agricultural productivity (Gautam and Anderson, 1999). Following liberalization and structural reforms in 1992, funding and delivery of agricultural extension services in Kenya became a mix of public and private arrangements.

The Government of Kenya (GOK) emphasizes the role of devolution in better service delivery. County

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governments are envisaged to be the primary centers for service delivery, economic expansion and good governance practices at the local level. Agricultural sector, particularly public extension service has been devolved to county government level in order to take the services closer to people and ensure they participate in improving the service delivery (Republic of Kenya, 2011). This presupposes that farmers at the county levels are aware of their responsibilities and expectations in the devolved extension system. Awareness means providing the public with detailed background information on policy issues regarding development. The aim is to empower the public to be aware of and understand global and national development concerns and the local and personal relevance of those concerns, and to enact their rights and responsibilities by effecting change for a just and sustainable world (Omolo, 2010). Thus, the right to information or the right-to-know enables citizens to make informed decisions on issues relating to their development.

Access to information has been widely recognized as a basic human right and an essential attribute of democracy. Meaningful public participation in development decisions requires that relevant information is provided in a timely manner, simple procedures and channels of access developed, cost to citizens be reasonable, and that it should be available across boundaries (Burton et al., 2006). Public demand for access to information is increasing, which is associated with use of freedom of information legislation and the revolution in information technology (O'Loughlin and Wegimont, 2007). In Kenya, articles 35(1&3) of the constitution recognize the right of every citizen to access information held by the state (Omolo, 2011).

The use of communication technology such as mobile phones, emails, satellite communications and geographic information systems has generated an extraordinary level of interconnectedness. This has helped to raise citizens' awareness of development issues such as climate change through presentations and dissemination of information. Media outreach, which is the main source of news and public information is a wide-reaching way to inform citizens on development matters. Public awareness and educational programmes have also been widely used to inform citizens. These approaches are considered to be more comprehensive and enable deepening of public awareness due to in-depth consultation (African Development Bank, 2007).

Among the objects and fundamentals of devolution in Kenya is enhancing participation of people in making decisions affecting them and the recognition of communities' rights to manage their own affairs (Republic of Kenya, 2011). This dimension of public participation is administrative centric and relates to the involvement of the public in decision making (Yang and Callahan, 2005). Existing literature show a considerable lack of awareness by farmers on various pertinent issues in developing countries. In India, up to 60% of farmers had limited awareness about climate change phenomenon and its impacts (Chakravarty et al., 2012). In Ghana, Laary et al. (2012) observed that some farmers were unaware of hazardous and inappropriate agrochemical products banned by government authorities and continued to use them without protective measures. In addition, some people in Uganda were not aware of rules and regulations for use of wetlands for improved food security and wetland integrity (Turyahabwe et al., 2017).

In Kenya, the Institute of Economic Affairs noted that there was limited awareness on costs of projects and disbursed amounts by the Constituency development Funds (CDF) program in many parts of the country (IEA, 2006). Similarly, another study showed that the majority of the respondents were not aware of the Local Authority Service Delivery Action Plan (LASDAP) that required local authorities to constructively engage local communities on matters of planning and development (LRFT, 2009).

Other studies on farmer awareness have focused on issues such as climate change (Mandleni and Anim, 2011), crop insurance (Oyinbo et al., 2013) and agrochemical safety (Laary, 2012). However, in the case of Kenya's agricultural extension devolution, little is known about local communities' awareness and understanding. Awareness is pertinent in the realization of the benefits of devolution related to community participation and the establishment of appropriate agricultural extension institutions (Kukamba, 2010).

METHODOLOGY

Sampling procedure and data collection

This study was conducted in Meru County of Kenya, which was purposefully selected due to its wide range of climatic conditions that favor a variety of agricultural enterprises (Monda, 2003). The study employed multi-stage cluster sampling approach to select respondents for the survey. This approach was preferred to other methods such as simple random sampling because as sampling procedure moves from secondary to the primary sampling unit, the sampling unit becomes more homogenous and the sampling error is minimized (Allen et al., 2002). A total of 288 respondents were randomly interviewed.

The data was collected using semi-structured questionnaires through a face-to-face interview. Face to face interview has its strength in that, immediate follow-up and clarifications are possible unlike alternatives approaches such as mail and telephone surveys, which are ridden with the challenge of high non-response (Mertens, 2005). The questionnaire captured data on farmer characteristics such as age, education, gender and income; land assets; farm enterprises; farmer's knowledge of agricultural extension devolution; use of agricultural extension and; access to institutional services including credit, markets and community group membership.

Theoretical framework

Various factors have been shown to influence awareness. For

Table 1. Sample characteristics.

Variable	Response (n = 288)
Household size (average number of adults)	3
Gender (% of female farmers)	58.3
Access to extension services in the past year (% of farmers)	72.9
Use of crop extension services in the past year (% of farmers)	68.4
Use of livestock extension services in the past year (% of farmers)	32.3
Days of attending farmer field in the past year (% of farmers)	54.9
Farmer field days are held at experimental station (% of farmers)	61.4
Average farm size (acres)	1.9
Percentage of farmers with title deed for their farms	55.9
Commercial farming of tea and bananas (% of farmers)	31.2
Percentage of farmers who sold crop produce	69.4
Livestock keeping (% of farmers)	84.7
Percentage of farmers in dairy farming	66.0
Percentage of farmers who sold milk in the past year	58.3
Average monthly income of the respondent (Kshs)	12,677
Average age of the respondent (in years)	41.2
Percentage of farmers with secondary education and above	55.2
Main occupation is farming (% of respondents)	86.1

Source: Authors' compilation from survey data (2013).

example in the studies of Bayard et al. (2007) and Mandleni and Anim (2011), education was found to negatively affect awareness on climate change. The reason given was that educated farmers had alternative income earning opportunities and thus are not concern much with agricultural issues. However, this was contrary to Deressa et al. (2009) who observed that education increased the probability of climate change awareness. Further, Okello et al. (2014) found the level of literacy to drive awareness of the ICT-based market information services.

Access to formal extension has also been found to positively influence awareness (Hassan and Nhemachena, 2008; Apata et al., 2009). Further, Kabubo-Mariana (2008) noted that married farmers and farmers who acquired land through inheritance have more knowledge on climate change. The possible influence of some of these variables on Kenyan farmers' awareness of extension devolution was explored in this study.

Given that the dependent variable in this study is discrete and dichotomous, aware of extension devolution or not, a binary logit model (Menard, 2002; Harrell, 2001) was considered to be most appropriate. The use of the log odds ratio provides a most simplistic description of the probabilistic relationship of the variables and hence more rich information can be drawn.

Empirical model estimation

The binary logit model for investigating factors that influence probability of farmers' awareness of extension devolution was modeled as follows:

$$Pr\{Aware_i = 1\} = \frac{exp\{\beta_0 + \beta_i x_i\}}{1 + exp\{\beta_0 + \beta_i \beta_i\}} \quad (1)$$

where *Aware* is the state of awareness of i^{th} farmer (1 = aware, 0 = otherwise); *x* denotes a vector of farmer and farm characteristic that are hypothesized to influence farmers' awareness of extension devolution; β represents the vector of parameters to be estimated. Marginal effects were estimated to measure the effects of changes in any explanatory variable on the predicted probability of awareness of agricultural extension devolution, *ceteris paribus*. The marginal effects for continuous variable and dummy-coded variables were computed following Equations 2 and 3, respectively.

$$\beta_m = [\partial(\beta_i x_i + \varepsilon_i)/(\partial\beta_i x_i)]\beta_i \quad (2)$$

$$\beta_m = Pr[Awaredvln_i = 1] - Pr[Awaredvln_i = 0] \quad (3)$$

The estimations were done using the NLOGIT version 4.0.

RESULTS AND DISCUSSION

Farmer characteristics

The socio-economic, demographic and institutional characteristics of the respondents are presented in Table 1. About 58% of the respondents were female and the mean age was 41 years, which shows that most of farmers are within the active and productive group in the community. Average farm size ranged between 0.25 and 20 acres with a mean of about 1.9 acres. This is consistent with the estimates of the African Development Bank Group that smallholder farming accounts for over 75% of agriculture production in Kenya (Salami et al., 2010).

The mean monthly income of the respondents is approximately Ksh 12,677 with about 55% of the

Table 2. Description of variables used in the binary regression model.

Variable	Expected signs
Attendance of farmer field days (1=yes, 0=no)	+
Farm size in acres	+/-
Title deed (1=yes, 0= otherwise)	+
Monthly income (Ksh)	+
Level of education (1=secondary level and above, 0=primary and below	+
Gender (1=male, 0=female)	+/-
Age in years	+/-

Source: Survey data (2013).

respondents having attained secondary level education and above. Perhaps, the low level of income among the farmers is due to low level of commercial farming as noted by Omiti (2006). In terms of tenure rights, 56% have land title deeds that signify security of land ownership and is a possible motivation for long term investment since the land can be used as collateral to access credit. Two-thirds of the farmers had access to crop extension services, while one-third had access to livestock advisory services.

Awareness of agricultural extension devolution

Although more than 60% of Kenyans voted for the devolved government system (IEA, 2010), less than half of the respondents were aware agricultural extension matters are expected to be handled at the county level. In order for the devolved governance system to achieve its objective, participation of the locals and accountability of the leaders is needed. Therefore, it is important to sensitize farmers on their role in achieving agricultural development.

The variables hypothesized to influence awareness of agricultural extension devolution and their expected signs are presented in Table 2. Most of the researches on awareness demonstrate that variables capturing access to extension service, farm size, tenure rights, income and education are expected to positively influence awareness. Simtowe et al. (2012) reported that farmers with larger land holdings have a higher chance of being exposed to improved varieties than those with smaller land holdings. On the other hand, it is possible that smaller land holdings mostly found in high potential areas are more productive, hence farmers may be more aware of agriculture related issues.

Tenure security may have a positive effect on awareness. This is supported by the findings of Asrat et al. (2004) who reported that tenure insecurity had a negative effect on awareness and willingness to pay for soil conservation measures. It has also been found that people with higher income and education are more likely to be aware and express a positive attitude towards organic product (Gracia and Magistris, 2007; Aryal et al., 2009). Further, Simtowe et al. (2012) showed that women had more awareness on improved pigeon pea varieties due to their higher propensity to being exposed to improved agricultural technology than men. Although, older farmers may be more experienced, which could have a positive effect on access to information, younger farmers may have a longer planning horizon, hence vibrant in searching for information (Faye and Deininger, 2005).

To ascertain the absence of multicollinearity between the explanatory variables used in the binary logit regression, variance inflation factors (VIF) were computed for each of the variables. The VIF was calculated as:

$$VIF_i = \frac{1}{1 - R_i^2} \quad (4)$$

Where, VIF_i is the variance inflation factor for the i^{th} explanatory variable and R_i^2 denotes the R^2 of the regression with i^{th} independent variable as a dependent variable. The VIF results are shown in Table 3 and according to Maddala (2000), variables that have VIF<5 are considered to have no multicollinearity.

Determinants of farmers' awareness on agricultural extension devolution

The parameters of binary logit regression were estimated using NLOGIT software and the results are shown in Table 4. The Chi square statistic of 219.38 (p< 0.1) showed that the model fitted the data well. The coefficients indicate the effect of each variable on the likelihood of a farmer being aware of agriculture extension devolution. On the other hand, the marginal effects show how a change in each variable influences the farmers' awareness.

The result shows that attendance to farmer field days is significant in influencing farmers' awareness of the

Table 3. Variance inflation factors.

Variable	VIF
Farm size	1.30
Possession of title deed	1.21
Education	1.16
Income	1.15
Farmer field days	1.12
Age	1.12
Gender	1.09
Mean VIF	1.15

Source: Survey data (2013).

Table 4. Binary logit estimates of factors influencing farmer's awareness on extension devolution.

Variable	Coefficient (β)	$\beta_{\text{p-value}}$	Marginal effect (β_m)	β_m p-value
Constant	-1.34 (0.16)***	0.00	-0.33(0.04)***	0.00
Field days	0.46(0.07)***	0.00	0.11(0.02)***	0.00
Farm size	-0.01(0.03)	0.84	-0.001(0.01)	0.84
Title deed	0.33(0.08)***	0.00	0.08(0.02)***	0.00
Income	0.0003(0.00005)***	0.00	0.00008(0.00001)***	0.00
Education	0.22(0.08)***	0.00	0.05(0.12)***	0.00
Gender	-0.02(0.07)	0.77	-0.01(0.12)	0.77
Age	-0.002(0.004)	0.61	-0.0005(0.0009)	0.61

***Indicate that the variable is statistically significant at 1%. Corresponding standard errors are shown in parentheses. Source: Survey data (2013).

extension devolution. More than two-thirds of farmers had access to extension services mostly from sources such as public agent, company agents and media. Farmers have also participated in Government spearheaded extension program such as Smallholder Horticulture Marketing Program (SHOMaP) (Republic of Kenya, 2007). Hence, this result can be explained by exposure to extension agents who might have played a role in informing farmers about agricultural extension devolution. Previous research on awareness (Hassan and Nhemachena, 2008; Apata et al., 2009) indicated that access to extension services had a strong positive influence on awareness on climate change. Further, attending agricultural technology lectures was found to influence the level of awareness of circular agriculture in China (Yang and Pan, 2014). Extension service forums appear to be a good tool for enhancing awareness on farming aspects.

Ownership of the farm with title deed increases the probability of farmers being aware of extension devolution. The literature shows that farm title deeds motivate farmers to do more permanent farm enterprises. Majority of the farmers in the survey were engaged in commercial farming of tea and bananas. These are more permanent investments which might have made the farmers to follow up with the updates and new issues concerning agricultural enterprises. This result agrees with the findings of Hassan and Nhemachena (2008) and Mandleni and Anim (2011) who reported that farmers with tenure security were more aware of climate change and invested in climate change adaptation methods.

Household income was found to have a significant positive effect on farmers' awareness on agricultural extension devolution. Majority of the respondents (86%) are farmers by occupation who grow crops and keep livestock for both domestic and commercial purposes (average quantity of milk sold per month is 127 L). It is therefore possible that a good percentage of respondent's income came from farm related enterprises hence expect them to be more aware of issues concerning their source of livelihood (agriculture). This is consistent with the observation of Munyua and Stilwell (2009) that people with higher income are likely to be more aware of new developments in different economic sectors. Formal education was found to have positive effect on farmers' awareness on the extension devolution. A higher level of education is expected to increase farmers' ability to process and use information (Turyahabwe et al., 2017; Saikia et al., 2013).

The marginal effect estimates reported in Table 4 show that attendance to field days has the highest influence (11%) on farmers' awareness on extension devolution, while possession of title deeds and formal education, respectively contributed to 8 and 5% influence on awareness. In Kenya, extension information is usually passed to farmers through on-station field demonstrations and information and communication technologies such as radio, mobile phones and television (Republic of Kenya, 2012).

CONCLUSION AND POLICY IMPLICATIONS

Considering the low level of awareness, there is need to develop more effective strategies to ensure that farmers understand how the decentralized extension system works. In addition, increased exposure of farmers to extension field demonstrations is essential in dissemination of agricultural information. Results show awareness level to be directly related to education, meaning that farmers who had attained a higher level of education were more aware of extension devolution. Considering that farmers and particularly small scale farmers generally have low levels of education (about half of the respondents had attained primary education at most), they may not be able to synthesize extension devolution from the broad information on devolution presented in unfamiliar languages. Hence, it may appear reasonable for the county governments to promote policies on publishing and airing extension devolution information in languages easily understandable by less literate farmers, particularly vernacular. Public and private investors could consider provision of incentives to radio and television channels that air information in vernaculars to slot in more programs on agricultural extension devolution. Land tenure security was as well found to significantly influence extension devolution awareness. Exclusive rights to access and use of farm lands may encourage more permanent investments in agricultural enterprises. Ultimately, improving awareness and understanding agricultural extension devolution would enable farmers to exercise their roles and rights in shaping extension service system, which could possibly contribute to development of the agriculture sector.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Full Length Research Paper

Effects of *Schinus terebinthifolius* extracts on the control of *Sitophilus* species in stored wheat grains

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The objective of this study is to evaluate the efficacy of *Schinus terebinthifolius* extract in controlling the insects of stored wheat. The experimental design was completely randomized with 10 replicates in a factorial scheme of 4 × 9, for extracts (control, aqueous extract, and hydro alcoholic extract of 5 and 15% of Brazilian pepper tree) and nine dates of evaluation (3, 6, 9, 16, 23, 30, 37, 44 and 51 days) after application. Ten weevil insects were inoculated into 200 g of wheat seed by applying 2 ml of each treatment. There is a control effect on the emergence and adults of *Sitophilus* species in wheat stored using *S. terebinthifolius* extracts. Up to the first nine days of storage, the hydro alcoholic extract (15%) has a relative efficacy of 35%. At 9 and 16 days of evaluation, hydro alcoholic extracts (5 and 15%) presented relative efficacy of 40%. At 44 and 51 days of evaluation, the aqueous extract shows relative efficacy of 40 to 60%. The aqueous extract appears to have an effect on the larvae and oviposition of the weevil observed by efficacy at 44 and 51 days. Hydro alcoholic extracts, on the other hand, show greater control over adults with efficacy until the 7th date.

Key words: *Triticum aestivum* L, storage, weevil, insecticidal action, Brazilian pepper.

INTRODUCTION

Wheat (*Triticum aestivum* L.) is one of the most produced cereals for human consumption, because it is used for flour production and the manufacture of various food products (Scheuer et al., 2011). It is also an excellent source of food for animals, provided in the form of feed, hay, silage or pasture. In rural properties, its cultivation ensures economical flow and sustainability of production, being integrated into schemes of crop rotation in no-

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tillage system (Nunes et al., 2011).

After harvest, wheat grains can lose their quality quickly if not stored properly, especially due to insect infestation, fungal contamination and metabolic processes that reduce germination and seed vigor (Da Silva et al., 2013). One of the main insects attacking wheat grains is *Sitophilus* species (Coleoptera: Curculionidae), commonly known as weevil (Plarre, 2010).

Weevil is the main pest of stored grains; it has high biotic potential, cross-infestation, high penetration capacity in the masses of grain, a high number of hosts. Both adult insects as well as larvae are capable of damaging grains (Gallo et al., 2002). The damages result from the penetration of the larvae into the seeds; it causes reduction of weight, germination, nutritional value of seeds besides contaminating grains with uric acid, causing unpleasant odor and reducing the quality of flour (Lorini, 2003).

Chemical control is mostly used for the protection of stored grains from the infestation of pests due to its effectiveness, low cost and easy handling. However, many synthetic insecticides do not exterminate pests and prevent reinfestation, which can result in resistance of insects, presence of residues in food, possibility of operators' intoxication and environmental contamination (Lorini, 2003; Jardim et al., 2009). Bearing in mind the losses arising out of the indiscriminate use of these products, associated with consumers' concern about food quality, research and studies were motivated regarding new techniques for controlling these pests and, among these, is the use of plant origin insecticides (Santos et al., 2007a; Souza and Trovão, 2009).

The plant extracts have countless advantages compared to the use of synthetic products. Since they are obtained from renewable resources, they are rapidly degradable, act without leaving residues in food, do not damage the ecosystem and are less toxic to humans (Olivo et al., 2008; Agnolin et al., 2010). The Brazilian pepper tree (*Schinus terebinthifolius*) has been widely studied and its extracts fight against insect pests (Santos et al., 2007a, 2013). This is due to the chemical constituents distributed in plants' parts, such as tannin, bioflavonoids, triterpernic acids, mono and sesquiterpenes present in leaves (Silva et al., 2011). Considering the insecticide potential of the *S. terebinthifolius* leaves and seeking to promote alternatives to the use of synthetic insecticides in storage pest control, this study aims to evaluate the efficacy of extract of Brazilian Pepper tree in the control of weevils (*Sitophilus* spp.) in stored wheat.

MATERIALS AND METHODS

The experiment was carried out in the Laboratory of Entomology, Regional University of Northwestern region of the State of Rio Grande do Sul (Unijuí). The experimental design was completely randomized with 10 replicates in a factorial scheme of 4 × 9, for extracts (control, aqueous extract, and hydro alcoholic extract of 5 and 15% of Brazilian pepper tree) and nine dates of evaluation (3, 6, 9, 16, 23, 30, 37, 44, and 51 days) after application under controlled environment (temperature ± 25°C, relative humidity of 70 ± 10% and photoperiod of 12 h).

The wheat grains (clean and dry) used in the experiment were packed in plastic bags and kept in a freezer at a temperature of -10°C for seven days to eliminate any infestations of insects coming from the field. After this period in the freezer, the grains were transferred to glass vials and maintained in the laboratory at ambient temperature for 10 days with the aim of achieving hygroscopic equilibrium (Coitinho et al., 2011).

The insects of *Sitophilus* spp. used in the experiments were obtained from a mass of stored grains. Such insects were kept in glass containers of 5 L, sealed with plastic cover pierced and covered internally with nylon screen net to allow gas exchange. After the confinement for 15 days to perform fecundity, the insects were discarded. The containers were stored until the first generation appeared. This procedure was performed by successive generations, to ensure the quantity of adults necessary for the implementation of the experiments, using the methodology of Coitinho et al. (2011).

The *S. terebinthifolius* (Anacardiaceae) leaves were collected in the municipality of Ijui in November 2015 (spring), where minimum, average and maximum temperatures were around 15, 17, and 23°C, respectively. The collections were obtained from the specimens of this species from different locations of the tree canopy in the morning. The aqueous extract was obtained with the Brazilian pepper tree leaves through the process of infusion (Farmacopeia, 2010). The water was heated on a magnetic stirrer until it boiled. It was placed on the leaves using the proportion of 200 ml/68 g between solvent/solute (Santos et al., 2007b). The solution obtained was filtered and bioassays were immediately carried out. The hydro-alcoholic extract was prepared by maceration with ethanol 92% (v/v), using the proportion of 68 g/200 ml (Santos et al., 2007b) between solute/solvent. The solution obtained was filtered and the solvent was eliminated in rotating evaporator under reduced pressure, with controlled temperature of 60°C during the entire procedure, giving rise to the hydro alcoholic extract from the *S. terebinthifolius* leaves (Farmacopéia, 2010). The extract obtained was suspended again in distilled water to obtain concentrations of 5 and 15%.

The bioassays were carried out in plastic containers of 1000 mL, in which 200 g of wheat grains were placed. 10 specimens of *Sitophilus* spp. from 0 to 15 days of age were inoculated. With a pipette, 2 ml of aqueous extract and hydro alcoholic extract of 5 and 15% of Brazilian pepper tree were applied on the grains in 10 repetitions per treatment. This quantity of the extract allowed the whole grain mass to get moistened with the extracts. The containers were sealed with fabric to prevent air from entering. After the treatment, the experimental units were placed in a laboratory with temperature ± 25°C, relative humidity of 70 ± 10% and photoperiod of 12 h during the 51 days of completion of the experiment.

For the evaluation of the number of insects (dead or alive), counting was carried out every three days (counted from the application of the extracts) in the first nine days and subsequently every seven days for the 51 days of the study. All insects that moved any part of their body were considered alive, even those that remained immovable and only moved slowly when stimulated. Also, the relative effectiveness of treatments was quantified, estimated by the formula modified by Gonzalo et al. (2003): E% = (NITe - NITr / NITe) × 100; where E% is the effectiveness; NITe is the number of living insects of the control; NITr is the number of living insects of the treatment.

The data were subjected to analysis of variance using the program GENES (Cruz, 2013) and test for comparison of means was done by Tukey test at $p<0.05$ probability of error. This was followed with adjustment of regression equations as a function of

Table 1. Summary of analysis of variance for the treatments of extracts of Brazilian pepper tree in the control of *Sitophilus* spp. in stored wheat in different dates for evaluation.

Variation source	Degrees of freedom	Mean square (Alive insects)
Dates	8	5932*
Treatments	3	1426*
Treatments × dates	24	1064*
Error	324	66
Total	359	-
Mean	12.81	-
CV (%)	63.28	-

*Significant at 5% probability of error.

Table 2. Test of means for extracts of Brazilian pepper tree in the control of *Sitophilus* spp. in stored wheat in different dates for evaluation.

Treatment	Date (days)								
	3	6	9	16	23	30	37	44	51
Aqueous	9.9^{Aa}	9.5^{Aba}	9.2^{ABCa}	8.0^{Da}	7.0^{Da}	7.0^{Da}	7.0^{Da}	8.4^{BCb}	10.1^{Ad}
Hydro alcohol	9.4^{Ca}	9.1^{Ca}	7.4^{Da}	6.3^{Da}	6.3^{Da}	6.3^{Da}	6.4^{Da}	16.5^{Bab}	83.8^{Aa}
Hydro alcohol 15%	8.6^{Ca}	6.5^{Da}	6.5^{Da}	6.5^{Da}	6.7^{Da}	6.7^{Da}	5.8^{Da}	12.5^{Bab}	34.6^{Ac}
Control	10.0^{Ca}	10.0^{Ca}	9.9^{Ca}	9.9^{Ca}	9.7^{Ca}	9.7^{Ca}	9.6^{Ca}	19.8^{Ba}	50.5^{Ab}

*Averages followed by the same upper case letters on the line and lower case letters in the column do not differ statistically among themselves at 5% of error probability.

the date of assessment in each treatment tested.

RESULTS AND DISCUSSION

In the analysis of variance (Table 1), there was a significant effect of the assessment periods and extracts, as well as the presence of interaction. Through the averages test (Table 2), there was no difference between treatments until the 37th day of evaluation (7th date). At the 44th day of evaluation, the hydro alcoholic extracts (5 and 15%) decreased the viability of insects compared to the control ($p<0.05$). However, in this date, the aqueous extract was more effective in the control of the weevils, presenting a lower number of living insects compared to the other treatments. At the last date of assessment (51st), the aqueous extract was the most effective in controlling the weevils, followed by the hydro alcoholic extract (15%) of Brazilian pepper tree.

It was found that after the 37th day of evaluation, there was emergence of insects. This is possibly due to the fact that the life cycle of the weevil is 34 days (Gallo et al., 2002). In that date, the aqueous extract presents greater efficacy, indicating the effect of control over the larvae and eggs of the insect. This result is similar to that observed by Maroneze and Gallegos (2009), which also proved the best efficiency of the aqueous extract based on *Melia azedarach* leaves in the development of the immature and reproductive stages of *Spodoptera frugiperda.*

The insecticide effect promoted by *S. terebinthifolius* may be related to the chemical structure of compounds present in extracts from its leaves. Santos et al. (2013) identified the presence of phenolic substances, such as flavonoids from the aqueous extract and ethyl acetate from the leaves of *S. terebinthifolius*. Whereas Guimarães et al. (2014), evaluating the repellant, insecticide and fago inhibitor effect of the extracts of pepper dedo-de-moça (*Capsicum baccatum*) over corn *Sitophilus zeamais* weevil, found that the aqueous extract of seeds of pepper dedo-de-moça was the one that presented the greatest potential insecticide compared to the alcoholic extracts of 2.5, 5 and 10%. This corroborates the results observed in this study.

The aqueous extract has an inflection point of 45 days, showing that after the 5th day of evaluation, the extract begins to lose the effect in the control of weevils (Table 3). Considering the hydro alcoholic extracts, there is adjustment of equation of degree 3. In the first assessment dates, effect of the extracts was not observed with high population of insects. Over a period of time, the treatments increased their effectiveness by having a maximum control of the weevils, which was 30 and 37 days for the hydro alcoholic extract of 5 and 15%, respectively. From this period, there was no more effect of extracts, because the number of living insects begins

Table 3. Adjustment of the regression equation considering the different dates of evaluation of wheat grains for the treatments of extracts of Brazilian Pepper in the control of *Sitophilus* spp.

Treatment	Equation	R^2 (%)
Aqueous	$y = 12.30 - 0.18x + 0.0017x^2$	79.65
Hydro alcohol 5%	$y = -18.96 + 3.33x - 0.10x^2 + 0.0008x^3$	91.05
Hydro alcohol 15%	$y = -0.29 + 0.99x - 0.031x^2 + 0.0002x^3$	92.49
Control	ns	ns

R^2 = coefficient of determination; ns: Not significant.

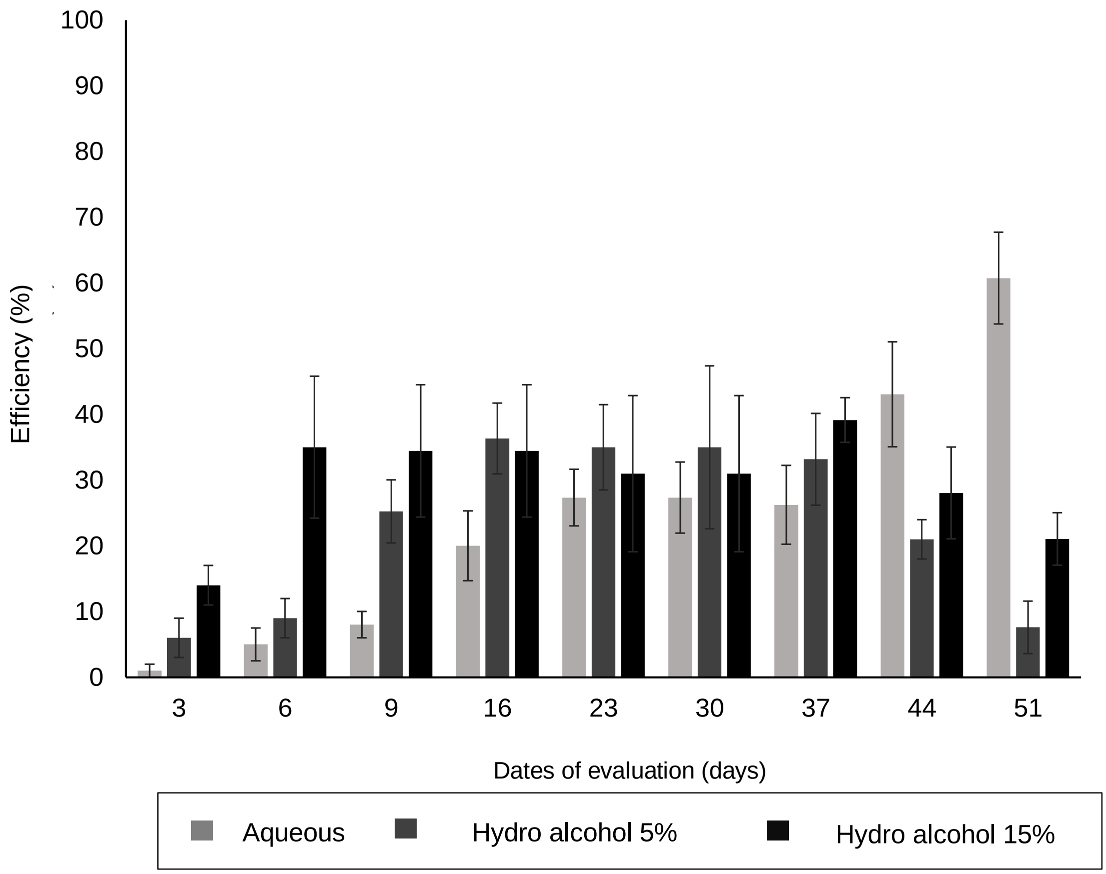

Figure 1. Relative efficiency (%) and standard deviations of the extracts of Brazilian pepper tree for the control of *Sitophilus* spp. in wheat grains.

to increase.

Regarding the relative effectiveness, the hydro alcoholic extract (15%) showed greater effect in the first nine days of storage, reaching 35% of effectiveness, in the initial period (Figure 1). After this date, the hydro alcoholic (5%) and aqueous extracts exhibited relative efficiency of 36 and 27%, respectively, in controlling the weevils; the effectiveness of the three excerpts remained very close until the 37th day of evaluation, when the life cycle of the weevil ends (Gallo et al., 2002). After the 37th day, there was emergence of new insects in the treatments period in which the aqueous extract stands out with relative efficiency of 61%. This indicates the effect of repellency, verified by the reduction or suppression of fecundation, in order to avoid reinfestation of insects in grain mass. For Coitinho et al. (2011), the smaller the infestation, the larger the repellency of the extract, a fact which favors the reduction of the fecundation and the number of insect's hatching. For Gullan and Cranston (2008), repellency is a reaction of the sensory system of the insect, when it is exposed to undesirable substances. The insects have chemical receptors located in various parts of their bodies and are responsible for assessing the conditions of the environment where they are running away in unfavorable conditions.

According to Pinto Júnior et al. (1997), an insecticide can only be considered effective if it presents minimum efficacy of 80%. However, several authors observed the insecticide effect of the extracts, but with relative efficiency below this rate. This is also observed by Souza and Trovão (2009) by testing the efficiency of neem extract on the mortality of *S. zeamais* in stored maize. In the present study, the effectiveness occurred at levels below 80% in all treatments and the aqueous extract presented the highest value in the experiment, reaching 61% of maximum effectiveness. What demonstrates that effectiveness of 80% may not be adequate when one considers extracts of plants or species are not well known, as *Schinus*, which controlled the population of weevils with relative efficiency than the index analyzed. Estrela et al. (2006) concluded that the effectiveness of the oil from *Piper hispidinervum* and *Piper aduncum* leaves in *S. zeamais* depends on the path of intoxication and the concentration of the oil applied. For Regnault-Roger (1997), the toxic effect of the extracts and essential oils involves many factors, including the location of entry of toxins, since they can be inhaled, ingested or even absorbed by the tegument of insects.

The purpose of demand by plants with insecticide properties should not always be focused on the insect's mortality, because mortality is only one of the effects. The mortality requires a higher concentration of the product, which requires a greater amount of raw material, which is often impractical from an economic and sustainable point of view. The main goal of natural products is to reduce or prevent oviposition, feeding and reproduction of insects (Vendramim and Castiglioni, 2000). In addition, it is important to develop studies concerning the action of plant extracts on the reduction of motor activity of the insect, time of life, change in sexual behavior in males, impotence and a reduction in reproduction of pheromone, and if there is acute mortality in other insects, in addition to *Sitophilus* spp. (Almeida et al., 2005). The insecticides actions of these extracts should provide new studies to determine the chemical substances responsible for this effect and evaluate the effective concentrations in terms of the storage period, persistence, mechanisms of action and other aspects necessary to make commercial use of these plant extracts to control this pest.

Conclusion

There is an effect of control over the emergence and adults of *Sitophilus* spp. in stored wheat using *S. terebinthifolius* extracts.

Until the first nine days of storage, the hydro alcoholic extract (15%) shows relative efficiency of 35%. From the 3rd to 7th assessment date, the hydro alcoholic extracts (5 and 15%) present relative efficiency of 40%. At the 8h and 9th date of assessment, the aqueous extract presents relative efficiency from 40 to 60%.

The aqueous extract seems to have an effect on the larvae and oviposition of the *Sitophilus* spp. observed by effectiveness on the 8 and 9th date. Whereas the hydro alcoholic extracts show better control over the adults with effectiveness until the 7th date.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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