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Full Length Research Paper

Market preferences for cowpea (*Vigna unguiculata* [L.] Walp) dry grain in Ghana

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Cowpea is an important crop in Ghana, serving as a major source of calories and high-quality protein for many people. An understanding of market preferences is necessary when targeting research and breeding efforts. This study makes use of data from 562 samples of cowpea dry grain collected from 91 markets across Ghana, analyzed using the hedonic model framework, to determine implicit prices of characteristics, including seed coat color, pattern, texture, the location of purchase, the gender of the vendor, seed size, and seed quality. The results indicate that market location and seed size are the most important characteristics regarding pricing. Improvements in infrastructure to facilitate transport of goods and dissemination of varieties with increased seed size could improve incomes for the smallholder farmers in Ghana who produce cowpea.

Key words: Characteristic value, consumer demand, hedonic pricing, seed coat, *Vigna unguiculata*.

INTRODUCTION

Cowpea (*Vigna unguiculata* [L.] Walp) (Fabaceae) is a warm-season legume, most often consumed as a grain, but also as a vegetable in the form of immature pods and leaves (Boukar et al., 2018). Cowpea is a versatile crop, with high drought and heat tolerance (Boukar et al., 2018). In Ghana, cowpea is grown across the country, with the areas of greatest production in the Northern Plains. The majority of production is by smallholder farmers, often as an intercrop with maize or millet (Ehlers and Hall, 1997). Ghanaian production of cowpea has been increasing: from 2013 to 2016 the volume of production increased from 200,404 to 206,378 metric tons per year (Ministry of Food and Agriculture, 2016). Early maturing varieties have been developed and disseminated by breeding programs to fill the “hunger gap” between June and August, between when farmers have sown their seeds but have not yet brought in the harvest (Fatokun et al., 2002).

Cowpea is a cash crop for small-holder farmers and a vital source of income, meaning that growing cultivars with more valuable characteristics could lead to increased income (Samireddypalle et al., 2017). However, for breeders to know which traits to target, it is necessary to determine those which are most desired by consumers. Seed coat color, pattern, and texture traits are important consumer-related traits in cowpea.

Previous research has shown that consumers make qualitative decisions about the acceptability, quality, and

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presumed taste of a product based on appearance and color (Jaeger et al., 2018; Simonne et al., 2001). Consumer preference for different cowpea seed coat traits varies across locations, with different seed coat traits desired in different places and for different uses: for example, lack of color for use as flour or solid brown for use as whole beans (Langyintuo et al., 2003; Mishili et al., 2009).

A major cowpea pest is the bruchid (*Callosobruchus maculates* [F.]) (Coleoptera: Bruchidae), a post-harvest pest, which infests stored grains and bore holes in the seeds. It is understood that consumers prefer seeds with lower levels of damage and expect discounted prices for damaged seeds (Langyintuo et al., 2004; Mishili et al., 2009). Until recently, most farmers did not have access to adequate storage methods and so regularly sold their product for low prices directly after harvest (Murdock and Baoua, 2014). The lack of proper storage resulted in high levels of infestation and lower quality seeds, with the number of holes in seeds available on the market increasing from the time of harvest in September (Langyintuo et al., 2004). To address the issues of bruchid infestation the Bean/Cowpea Collaborative Research Support Program developed the triple bag technology known as Purdue Improved Cowpea Storage (PICS) which can protect seeds at low cost (Murdock and Baoua, 2014). It has recently been reported by Ibro et al. (2014) that 64% of cowpea in the West African countries of Burkina Faso, Niger, and Nigeria was stored in hermetic containers, including PICS bags, which reduce the incidence of infestation. Similar levels of adoption in Ghana might also be expected.

The consumer goods characteristics model, a hedonic pricing model, is a linear model for estimating consumer demand for specific traits based on quality which was developed by Ladd and Suvannunt (1976). Using the model, it is possible to determine the implicit value of the attributes of a good. This model is widely applicable and has been used to analyze prices of a wide variety of goods, including ecosystems (Czembrowski and Kronenberg, 2016), cloud computing services (Wu et al., 2018), used cars (Prieto et al., 2015), and origin country of imported meat (Hussein and Fraser, 2018). Previous analyses of the characteristics of cowpea dry grain and how those relate to price have been done within and comparing between African countries (Faye et al., 2004; Langyintuo et al., 2004; Mishili et al., 2009; Mundua et al., 2010). However, those which have included Ghana have only examined markets either in the north (Langyintuo et al., 2004) or south (Mishili et al., 2009) of the country. To date, no country-wide analysis of market preferences has been performed for Ghana. While it has been reported that cowpea is transported from inland production regions of West Africa to coastal regions (Langyintuo et al., 2003), the effects of such movement on consumer prices have not been examined. In this study, market preferences of Ghanaian consumers for cowpea dry grains are examined to determine which traits have the greatest implicit values.

MATERIALS AND METHODS

Sample and data collection

562 samples of cowpea were collected from 91 markets distributed across the ten regions of Ghana in July and August 2018 (Figure 1). In each region, samples were purchased from vendors in local markets where consumers purchase cowpea for end-use consumption. At the time of purchase, the location, gender of the vendor, and price paid were noted. During July and August 2018, the exchange rate was about 4.80 Ghanaian Cedi (GHS) to 1.00 United States Dollar (USD). In the lab, the price per kilogram of the purchased seed was determined and seed coat characteristics including color, pattern and texture were noted. Color was defined by the presence of a pigment in the seed coat, including black, brown, red and purple. Pattern was defined by how the observed pigmentation was distributed on the seed coat, including eye, solid coat, speckled, and mottled, among others. Three 100-seed subsamples were taken from each sample. In these subsamples, 100 seed weight and number of holes per 100 seeds were noted. For analysis, the average of the three values was used, rounded to the first decimal place. Due to mixtures in the seeds purchased, for analysis the most prevalent seed coat traits (>75% of seeds in the sample) were used as when collectors reported the type of seed purchased they ignored traits held only by a minority of the sample. To determine the latitude and longitude of each market, Google Maps (maps.google.com) was used.

Hedonic analysis framework

Implicit values of the observed characteristics of seeds were analyzed through the use of a hedonic analysis framework, originally developed by Ladd and Suvannunt (1976). For this analysis, a simplified equation used by Langyintuo et al. (2004) and Mishili et al. (2009) was used, which takes the form of:

$$P_C = \sum_{j=1}^{m} X_{Cj}\,\beta_{Cj} + \epsilon$$

where P_C is the price of cowpea, X_{Cj} is the quantity of cowpea characteristic *j*, β_{ij} is the regression coefficient (implicit price) of characteristic *j*, and ϵ is a normally distributed random error. Analysis was done using the linear regression function in R. Tested factors included seed coat color, pattern, and texture, seed weight, infestation levels, purchase location, and vendor gender.

RESULTS

Range of characteristics

The full range of characteristics of the collected samples can be found in Table 1. Across Ghana, there is high variability in 100 seed weight, ranging from 7.3 g to 40.1 g, with a mean of 17.4 g and a standard deviation of 6.4 g. The number of bruchid holes per 100 seeds ranged

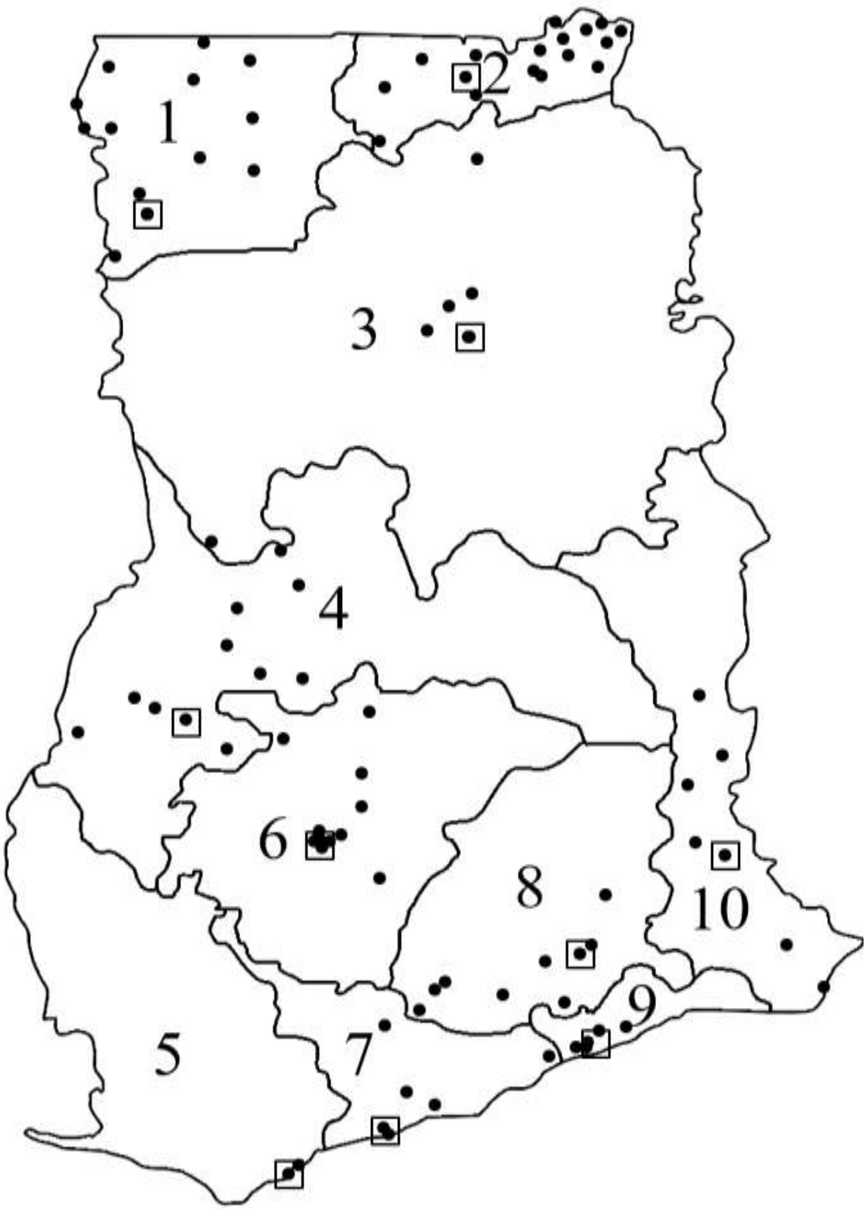

Figure 1. Location of markets at which samples were purchased. Black dots indicate market locations. Squares indicate regional capitol city locations. Map available from Wikimedia Commons. 1 = Upper West, 2 = Upper East, 3 =Northern, 4 = Brong Ahafo, 5 = Western, 6 = Ashanti, 7 = Central, 8 = Eastern, 9 = Greater Accra, 10 = Volta.

from 0 to 69.0, with a mean of 6.3 and a standard deviation of 3.4. The price per kilogram of seeds ranged from 1.8 Ghanaian Cedis (GHS) to GHS 23.3, with a mean of GHS 6.1 and a standard deviation of GHS 2.7. The most expensive sample, from Nsawam market in the Eastern region is most likely an outlier. The next most expensive sample had a price per kilogram of GHS 18.7. Most cowpea vendors were women. Of those samples for which the vendor's gender was recorded 95.9% (493) were sold by women and 4.1% (21) were sold by men. The most common pattern was the presence of an eye, which was in 66.7% (375) of the samples, followed by 19.8% (111) of the samples with a solid coat, 5.5% (31) with a speckled coat, 4.1% (23) with a mottled coat, and 3.9% (22) with other seed coat patterns. Seeds which had a clearly defined eye and additional pigmentation were considered as having an eye pattern for this analysis. 69.8% (392) of the samples had rough seed coats, 30.2% (170) had smooth seed coats. 49.3% (277) had black coloring, 15.8% (89) had brown coloring, 7.1%

Table 1. Statistics of quality and price metrics of cowpea in Ghana.

Region	#Samples	100 seed weight (g)		# holes / 100 seeds			Price	Vendor
		Average	σ	Average	σ	Average	(GHS/kg) σ	(F/M/NR)
Ashanti	77	17.4 (8.9-29.6)	5.5	3.2 (0-23.7)	4.0	5.9 (4.0-10.3)	1.4	75/2/0
Brong Ahafo	83	16.2 (8.5-37.1)	6.1	6.3 (0-50.7)	2.2	5.2 (2.9-8.1)	1.3	79/4/0
Central	35	19.9 (9.6-40.1)	7.0	6.5 (0-33.7)	8.6	6.9 (3.7-18.7)	3.4	31/4/0
Eastern	80	19.6 (9.5-36.4)	7.5	5.8 (0-41.0)	7.8	7.3 (2.3-23.3)	3.5	62/3/15
Northern	55	15.3 (7.3-29.8)	5.7	1.0 (0-11.7)	2.2	5.4 (2.6-8.5)	1.4	55/0/0
Greater Accra	38	19.6 (9.3-35.1)	6.7	2.9 (0-15.3)	3.8	11.0 (7.8-17.5)	2.6	30/8/0
Upper East	75	16.4 (9.0-30.1)	4.6	1.7 (0-21.7)	3.2	5.4 (3.1-12.4)	1.7	75/0/0
Upper West	52	15.1 (9.2-29.1)	5.1	5.8 (0-30.3	3.8	4.3 (1.8-12.7)	2.2	19/0/33
Volta	47	18.2 (8.7-35.9)	6.5	5.4 (0-69.0)	10.9	5.7 (2.6-9.7)	1.5	47/0/0
Western	20	20.2 (10.5-38.8)	9.8	4.6 (0-15.7)	3.9	5.1 (3.6-9.5)	1.6	20/0/0
Ghana	562	17.4 (7.3-40.1)	6.4	6.3 (0-69.0)	3.4	6.1 (1.8-23.3)	2.7	493/21/48

The ranges of values are in parentheses. Mean weight and number of holes per 100 seeds values are averaged from three subsamples of a market sample. All values are rounded to the nearest single decimal. GHS = Ghana Cedi, F = female, M = male, NR = not recorded.

(40) had red, brown, and purple coloring, 6.9% (39) had red and brown coloring, 6.9% (39) had red and purple coloring, 1.1% (6) had purple coloring, and 0.4% (2) had purple and brown coloring. The remaining 70 samples consisted of mixed seeds of various colors and so were considered missing data to avoid biasing the analysis.

Hedonic pricing

The hedonic pricing indicates that 26% of price variability of cowpea in Ghanaian markets is due to the tested characteristics. Table 2 shows the effects of different characteristics. Table 3 shows the analysis of variants table. All price effects are relative to a rough black seed with an eye pattern sold by a female vendor. The most significant effects were market location, seed weight and vendor gender, with a minor effect by the seed coat pattern. Prices decreased by GHS 0.33 per degree north and GHS 0.90 per degree west. For larger seeds, consumers were willing to pay an additional GHS 0.09 for each gram increased per 100 seeds. Seed coat color, pattern, and texture traits were not significant except for a seed with the mottled pattern, for which consumers were willing to pay an additional GHS 2.07. The price for seeds purchased from a male vendor was increased by GHS 1.78. The effects of number of bruchid holes per 100 seeds, seed coat color, the eye pattern, the speckling pattern, and texture were not significant.

DISCUSSION

The price of cowpea was uniform across all markets, with a mean price of GHS 6.1 per kg and a standard deviation of GHS 2.7. However, this finding was skewed by the much higher prices from the Greater Accra Region, which had an average price of GHS 11.0, a 75% increase over the country-wide average. The increased price in the capitol follows the trend reported by Mishili et al. (2009), but the difference is much more marked. The higher prices parallel the higher cost of living in general in Accra compared to other parts of the country (”Cost of living” 2018), as well as the fact that cowpea is not produced in the area and so the supply must be imported. During the collection period, it was noted by author Ira A. Herniter that street food in Accra cost twice as much compared to other locations, including both major cities like Kumasi,

Table 2. Estimated coefficients for cowpea seeds.

Coefficient	Estimate	Std. Error	t value	Pr(>|t|)	Significance
(Intercept)	7.95	0.84	9.49	< 2e-16	****
Location					
North (°)	-0.33	0.06	-5.16	3.82E-07	****
West (°)	-0.90	0.13	-6.94	1.46E-11	****
Vendor Gender					
Male vendor	1.78	0.59	3.03	2.57E-03	***
Seed coat color					
Brown	-0.23	0.38	-0.60	5.51E-01	-
Purple	-0.87	1.07	-0.82	4.15E-01	-
Purple Brown	0.12	1.72	0.07	9.45E-01	-
Red Brown	0.42	0.45	0.92	3.57E-01	-
Red Purple	0.28	0.52	0.54	5.91E-01	-
Red Purple Brown	0.63	0.59	1.06	2.92E-01	-
Seed coat pattern					
Full Coat	0.47	0.82	0.57	5.67E-01	-
Mottled	2.07	0.91	2.27	2.37E-02	**
Other	-0.92	0.57	-1.62	1.06E-01	-
Speckling	0.68	0.88	0.77	4.42E-01	-
Smooth	-0.59	0.76	-0.78	4.36E-01	-
Other seed characteristics					
100 seed weight (g)	0.09	0.02	4.07	5.62E-05	****
Holes per 100 seeds	-0.02	0.02	-1.30	1.96E-01	-

The intercept indicates a sample with the following characteristics: seeds with black coloring and an eye pattern, purchased from a female vendor. Probability codes: 0 = ****, 0.001 = ***, 0.01 = **, 0.05 = *. System R^2 = 0.26.

Table 3. Analysis of variants table for cowpea seeds.

Coefficient	Df	Sum Sq	Mean Sq	F value	Pr(>F)	Significance
Gender of Vendor	1	104.64	104.64	19.19	1.49E-05	****
100 seed weight (g)	1	211.27	211.27	38.74	1.14E-09	****
Holes per 100 seeds	1	0.00	0.00	0.00	9.79E-01	-
Seed Coat Color	6	32.70	5.45	1.00	4.25E-01	-
Seed Coat Pattern	4	44.43	11.11	2.04	8.83E-02	*
Seed coat texture	1	0.50	0.50	0.09	7.63E-01	-
North	1	166.82	166.82	30.59	5.51E-08	****
West	1	262.45	262.45	48.13	1.46E-11	****
Residuals	434	2366.58	5.45	-	-	-

Significance codes: 0 = ****, 0.001 = ***, 0.01 = **, 0.05 = *.

the capitol of the Ashanti region, and smaller towns like Bawku, in the Upper East region. Market location had a large effect on cowpea price. The price decreased in markets further north and west. The major areas of cowpea production in Ghana are in the north of the country. Indeed, the lowest average price could be found in the Upper West region, which produces the most cowpea in Ghana.

The dominance of women as petty traders in markets observed in this study, where 95.9% of samples were purchased from women vendors, conforms to previous research about vendors in Ghana. Both Langyintuo et al. (2004) and Mishili et al. (2009) reported that women are primarily the market vendors in Ghana. Indeed, in observations of markets in Ghana by the authors, it was noted that the majority of vendors of any type were women. Men are much more highly involved in the wholesale business of cowpea grains.

The two most common patterns observed in samples were the presence of an eye, where pigmentation is restricted to the area around the hilum and full coat pigmentation. Previous studies on consumer preferences for cowpea seed traits have quantified the value of the eye pattern but make no distinction between types of eye (Faye et al., 2004; Langyintuo et al., 2004; Mishili et al., 2009; Mundua et al., 2010). Of the observed seed coat traits, only the mottled pattern had a significant effect on price. This could be due to the market already accounting for consumer preferences. For example, no samples consisted of seeds with black color and a full coat pattern. Indeed, it is common knowledge that no market exists for such seeds in West Africa.

The size of cowpea, as measured by 100 seed weight, averaged 17.4 g, with a standard deviation of 6.4 g. In contrast, cowpea sold in the United States has a 100 seed weight of 20-25 g (P. A. Roberts 2018, personal communication, 28 August). Since cowpea serves as a cash crop for many farmers, increases in both yield and seed size can have major positive effects on farmer income. It should be noted, however, that the observed seed size is higher than previously reported seed sizes of 12.2 g per 100 seeds in the north of Ghana (Langyintuo et al., 2004) and 14.4 g per 100 seeds in southern Ghana (Mishili et al., 2009). The observed increase in seed size could be due to the release and adoption of improved lines since the previous studies. Almost all the largest seeds, those with 100 seed weight greater than 30 g, for which sources were reported or which had names indicating the source, came from Nigeria. Nigeria is the largest producer of cowpea in the world, outputting 58% of worldwide production (IITA, 2018). In contrast, seeds which were purported to come from neighboring countries, such as Burkina Faso and Togo, had seeds similar in size to those produced in Ghana.

It is common knowledge that consumers are adverse towards bruchid holes in seeds, seeing those with holes as lower quality. The average number of holes observed across all of Ghana was 6.3 per 100 seeds, with a standard deviation of 3.4 holes. This indicates a low tolerance for bruchid holes across Ghana. Indeed, during collection of the samples, vendors were seen sorting through their stock to remove seeds with holes. Previous studies reported much higher levels of infestation than observed here. Langyintuo et al. (2004) reported an average of 13.0 holes per 100 seeds in northern Ghana while Mishili et al. (2009) reported an average of 12 in southern Ghana. No region examined in this study had levels of infestation comparable to these levels. The decrease in infestation levels may be due to the use of improved storage techniques, including the use of triple bag and chemical storage systems (Ibro et al., 2014). It is notable that the number of holes per 100 seeds was not found to have any significant effect on price. This may be due to the relatively low incidence of insect damage. Further, the collection period was in August and September, before the harvest, so the incidence of holes would be expected to be the greatest at this time.

While other analyses of cowpea prices were able to describe over 90% of observed price variation, the present analysis accounts for only 26% of the variability. One possible cause could be differences in sample collection methods. Previous studies collected samples at a small number of locations (3 to 5) over the course of several years, while the present study collected from many locations over the months of July and August. This period is referred to as the “hunger period” as it is between when the seeds have been sown, but the harvest has not yet been brought it. This shortage of supply causes prices to rise, especially in the south of the country where the supply must be imported, so it is the time of year with the highest prices. Additionally, the low number of samples collected for this study (562) compared to previous ones (over 500 per market) likely contributes to the low R^2 value. To better understand the national market, future studies should combine the approaches and collect both widely and over a longer time.

Conclusions

This study uses samples collected from 91 markets spread across the ten regions of Ghana in July and August 2018 to estimate the value of certain characteristics to consumers in Ghana. Consumers prefer large seed size and price is determined mostly by location of purchase. The specific pigments present on the seed coat, seed coat texture and most of the patterns of the pigmentation are of low importance to Ghanaian consumers. The location effect indicates that increased profits for smallholder farmers could be achieved through dissemination of varieties with larger seeds and with more developed infrastructure to allow smooth transport of goods.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Rhizosphere yeast *Torulaspora globosa* with plant growth promotion traits and improvement of the development of tomato seedlings under greenhouse conditions

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Yeasts are an interesting group of microorganisms, which occur naturally in soil and on plant surfaces. Few studies have analysed their potential as plant growth promoters. Thus, the aim of this study was to evaluate the indole acetic acid (IAA) production and phosphate solubilization by the yeasts *Torulaspora globosa* (CCA5S51 and CCA5S55), *Meyerozyma guilliermondii* (CCA3C98), and *Rhodotorula mucilaginosa* (CCA2F32), and the influence of *T. globosa* (CCA5S55) in the development of tomato seedlings. The results showed that *T. globosa* strains present both plant growth promotion traits (IAA production and phosphate solubilization). The strains of *T. globosa* (5S51 and 5S55) showed high IAA production (641 and 669 µg.ml-1, respectively) after 48 h of incubation, while *Rh. mucilaginosa* produced 406 µg.ml^{-1} of IAA after 120 h. The strains CCA5S55 and CCA5S51 could also solubilize 47 and 35% of tricalcium phosphate in the medium, respectively, after 12 days of incubation; whereas *M. guilliermondii* (CCA3C98) solubilized only 10% of the tricalcium phosphate after 12 days. The inoculation of tomato seedlings with *T. globosa* stimulates the plant growth; root height was statistically superior when the higher cell concentration was inoculated. The root dry weight was enhanced with addition of glucose and tryptophan. The conclusion is the yeast species *T. globosa* is able to produce IAA in the presence of tryptophan and also solubilize phosphate *in vitro*. The inoculation of tomato seedlings promoted its development. The cell concentration and the addition of glucose and tryptophan must be evaluated in details to attain optimized yields.

Key words: indole acetic acid (IAA) production, phosphate solubilisation, yeast as plant growth promoter.

INTRODUCTION

The soil and plant ecosystem is a complex environment with a very high microbial diversity. It is characterized by different functional types; the interplay of which is fundamental to different processes, including nutrient cycling, protection, and stimulus to the development of plants. However, the current agricultural practices employ chemical compounds such as mineral fertilizers and chemical defensives, which disrupt the balance of the microbial ecosystem by decreasing biodiversity and consequently diminishing the resilience of the agricultural environment. However, the use of these products is essential, since the plant production process is a vicious cycle: highly dependent on inputs that support the yield while damaging the environment (Zhang et al., 2018).

The role of microorganisms in the soil and plants remains unclear. Several studies on rhizosphere microorganisms, especially rhizobacteria, have reported encouraging results in the promotion of plant growth and as biological agents in control of phytopathogens and plagues (Vejan et al*.,* 2016; Liu et al*.,* 2017). These studies highlight the potential of rhizosphere microorganisms to support various economically important crops. This group of microorganisms, known as Plant Growth Promoting Microorganisms (PGPM), can produce compounds capable of stimulating plant development through various mechanisms, such as the production of plant hormones, antagonism against pathogens, induction of plant resistance, and mineral solubilization (Gray and Smith, 2005).

The phytohormone auxin, through its main representative indole acetic acid (IAA), is a class of plant hormones produced by PGPM. IAA is known to stimulate a rapid and sustained response by plants, mainly by promoting the elongation of cell roots (Cleland, 1990). Several microorganisms, including bacteria (Khalid et al., 2004; Ahemad and Kibret, 2014), filamentous fungi (Floch et al., 2003; Gravel et al., 2007; Hoyos-Carvajal et al., 2009), and yeast are able to produce IAA and significantly influence plant growth and development (El-Tarabily, 2004; Nutaratat et al., 2014).

There is also a great interest in the application of PGPM as biofertilizers to eventually phase out chemical fertilizers. These microorganisms can improve plant nutrition by increasing the availability of nutrients in the soil, by the solubilization of inorganic compounds via organic acids production (Wei et al., 2010) and enzymatic mineralization (Alori et al*.,* 2017).

Yeasts are unicellular fungi, which are often used in biotechnological processes. This microbial group can also be found in the rhizosphere and on the surface of plants, but in a low number compared to bacteria and filamentous fungi (Yurkov, 2018). Hence, little is known about their role in this ecosystem, with few studies available in the literature. A wide variety of yeast species has plant growth promotion traits (Cloete et al., 2009; Limtong et al., 2014; Nutaratat et al., 2014), including the control of phytopathogens (El-Tarabily, 2004; Sansone et al., 2005; El-Tarabily and Sivasithamparam, 2006; Rosa et al., 2010; Korres et al., 2011; Platania et al., 2012; Yu et al., 2012), production of phytohormones (Nassar et al., 2005), phosphate solubilization (Falih and Wainwright 1995; Alonso et al., 2008; Hesham and Mohamed, 2011; Mundra et al., 2011), nitrogen and sulfur oxidation (Falih and Wainwright, 1995), production of siderophores (Sansone et al., 2005), and the stimulation of root colonization by mycorrhizal fungi (Vassileva et al., 2000; Alonso et al., 2008).

Therefore, it acknowledges that the present challenge is to expand our knowledge about the yeast niches in soil and their association with plants. This study aims at investigating the mechanism of action of this microbial group in assisting plant production, contributing to the balance of the ecosystem, and minimizing the use of toxic chemicals in the agriculture.

MATERIALS AND METHODS

Yeasts strains

The yeasts to be evaluated were isolated from the rhizosphere, stem, and leaf of sugarcane and maize. The isolation of strains was realized, during the prospection of yeasts as biological control agents against phytopathogens. The strains were identified by molecular taxonomy based on the analysis of the D1/D2 domain of the large subunit (LSU) rRNA gene. The strains were screened for their ability to produce IAA and solubilize phosphate *in vitro*, and four yeast strains (*Torulaspora globosa*–strains CCA5S51 and CCA5S55; *Meyerozyma guilliermondii*–strain CCA3C98 and *Rhodotorula mucilaginosa*–strain CCA2F32) were selected (data not published) (Table 1).

Quantitative indole acetic acid (IAA) production

The strains CCA5S51, CCA5S55, and CCA2F32 were evaluated to produce IAA. The assay was carried out in Erlenmeyer flasks (500 mL) containing 200 mL of liquid culture medium Potato Dextrose (BD) (200 mL of potato broth and 20 g of dextrose per litre, pH unadjusted) with or without 0,1% tryptophan solution. The flasks were inoculated with 1 ml of yeast cell suspension (5×10^4 cells.ml^{-1}) and incubated on a shaker at 25°C, 160 rpm for seven days. Samples were collected every 24 h. For quantification of IAA, each sample was centrifuged at 1000 × g for 3 min and 1.5 ml of the culture supernatant was pipetted into microtubes followed by addition of 1.5 ml of the Salkowski reagent (0.5 M ferric chloride and 35% perchloric acid) (Gordon and Weber, 1951). The tubes were kept for 30 min at room temperature, after which the development of pink colour revealed the presence of IAA. The

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Table 1. Characteristics of yeast strains selected and plant growth promoting traits.

Strain code	Source	Identification	GenBank accession no. (closest species)	AIA production	Phosphate solubilization
CCA5S51	Sugar cane rhizosphere	*Torulaspora globosa*	KY109864.1	+	+
CCA5S55	Sugar cane rhizosphere	*Torulaspora globosa*	KY109864.1	+	+
CCA2F32	Sugar cane leaf	*Rhodotorula mucilaginosa*	FJ515212.1	+	-
CCA3C98	Maize stem	*Meyerozyma guilliermondii*	MG323878.1	-	+

intensity of the colour was analysed using a spectrophotometer at a wavelength of 530 nm. To quantify the amount of IAA, a standard curve was prepared based on the optical density (OD) of IAA solutions at known concentrations. For evaluation of yeast growth, yeast cells in every sample were counted by microscopy in a Neubauer chamber, and the results were expressed as the number of cells per millilitre (Camacho-Fernandez et al., 2018).

Quantitative phosphate solubilization

The strains CCA5S51, CCA5S55, and CCA2F32 were evaluated as tricalcium phosphate solubilizers. The assay was performed in an Erlenmeyer flask (125 mL) containing 50 mL of liquid medium Pikovskaya (0.5 g.l^{-1} yeast extract, 10 g.l^{-1} glucose, 0.5 g.l^{-1} ammonium sulphate, 0.2 g.l^{-1} potassium chloride, 0.1 g.l^{-1} magnesium sulphate, 0.0001 g.l^{-1} manganese sulphate, 0.0001 g.l^{-1} iron sulphate), with or without tricalcium phosphate (5 g.l^{-1}). The flasks were either inoculated with the yeast strains or left uninoculated, depending on the treatments. The experiment was carried out in three sets each for the three treatments (T1: yeast + medium + tricalcium phosphate; T2: medium + tricalcium phosphate; T3: medium + yeast). Treatments T1 and T3 received the inoculation of 1 ml suspension with 5×10^4 yeast cells.mL^{-1}. The flasks were incubated for 12 days at 25 °C and 160 rpm, with sampling every 72 h. The evaluation involved the quantification of soluble phosphate in the culture medium, following the method described by Strickland and Parsons (1960) using the molybdenum blue colorimetric method. The intensity of the blue colour of the medium was assessed using a spectrophotometer at a wavelength of 880 nm. A standard curve was prepared using known concentrations of soluble phosphate. The pH of the medium was also determined using a digital pH meter (MS Tecnopon® mPA210).

Pot experiment under greenhouse conditions

The strain 5S55 was selected for inoculation of tomato seedlings. The inoculum was produced in YEPD medium (10 g.l^{-1} yeast extract, 10 g.l^{-1} peptone, and 20 g.l^{-1} dextrose) in a shaker at 160 rpm, 30°C for three days. Twelve different types of treatments, consisting of combinations of three concentrations of cells (1×10^8, 3×10^8, and 9×10^8 cells.plant^{-1}), presence or absence of glucose (20 g.l^{-1}), and the presence or absence of tryptophan (0,1%) were evaluated. The control group consisted of plants treated with sterile water. The inoculation of tomato seedlings (30-day-old plants) was carried out after transplanting to 5 L plastic pots containing non-sterile commercial organic substrate. The inoculum was sprayed near the root. The plants were maintained in a greenhouse for 45 days, while they were being evaluated for their height and root length (in centimetres) and dry weight (in grams) of shoot and root. The experimental design was completely randomized, with ten repetitions for each treatment. All the results were analysed using Analysis of Variance (ANOVA) and comparison of means by Tukey's test at 5% significance level using the statistical program STATISTICA 6.0 (Statsoft, 2001).

RESULTS AND DISCUSSION

The strains of *T. globosa* and *Rh. mucilaginosa* produced a large quantity of IAA (Figure 1). *T. globosa* (strain CCA5S55) showed maximum production of IAA (669 μg.ml^{-1}) after 48 h of incubation, which was statistically similar to the strain CCA5S51 (641 μg.ml^{-1}) after the same incubation period. The IAA production by *T. globosa* was statistically superior to that of *Rh. mucilaginosa* (CCA2F32), which produced 407 μg.ml^{-1} after 144 h of incubation.

M. guilliermondii (CCA3C98) was unable to produce IAA *in vitro* under the established conditions of this study. In contrast, Limtong and Koowadjanakul (2012) reported that an isolate of *M. guilliermondii* (strain LM120) grown in YEPD culture medium supplemented with tryptophan could produce 68.1 μg.ml^{-1} of IAA after seven days of incubation. Nakayan et al. (2013) and Nutaratat et al. (2014) also observed that IAA was produced by the isolates of *M. guilliermondii* (strains CC1 and DMKU-RP168) under the same conditions. These studies reported an average production of 10.6 μg.ml^{-1} after five days and 45 μg.ml^{-1} after seven days of cultivation, respectively. Thus, our results highlight two important issues related to the production of IAA by yeasts: first, varying results are obtained from diverse strains of the same species, especially if they are isolated from different environments (soil, rhizosphere, or phyllosphere); secondly, the specific conditions necessary for the IAA production, such as sources of nutrients and pH of the medium may influence the production significantly, even preventing it (Apine and Jadhav, 2011; Scarcella et al., 2017).

The production of IAA by all strains was dependent on tryptophan. This is common among yeasts, as reported by Nassar et al. (2005), who evaluated 24 yeast strains for IAA production in the presence and absence of tryptophan and observed production only in the presence of the amino acid. Contrasting results were reported by

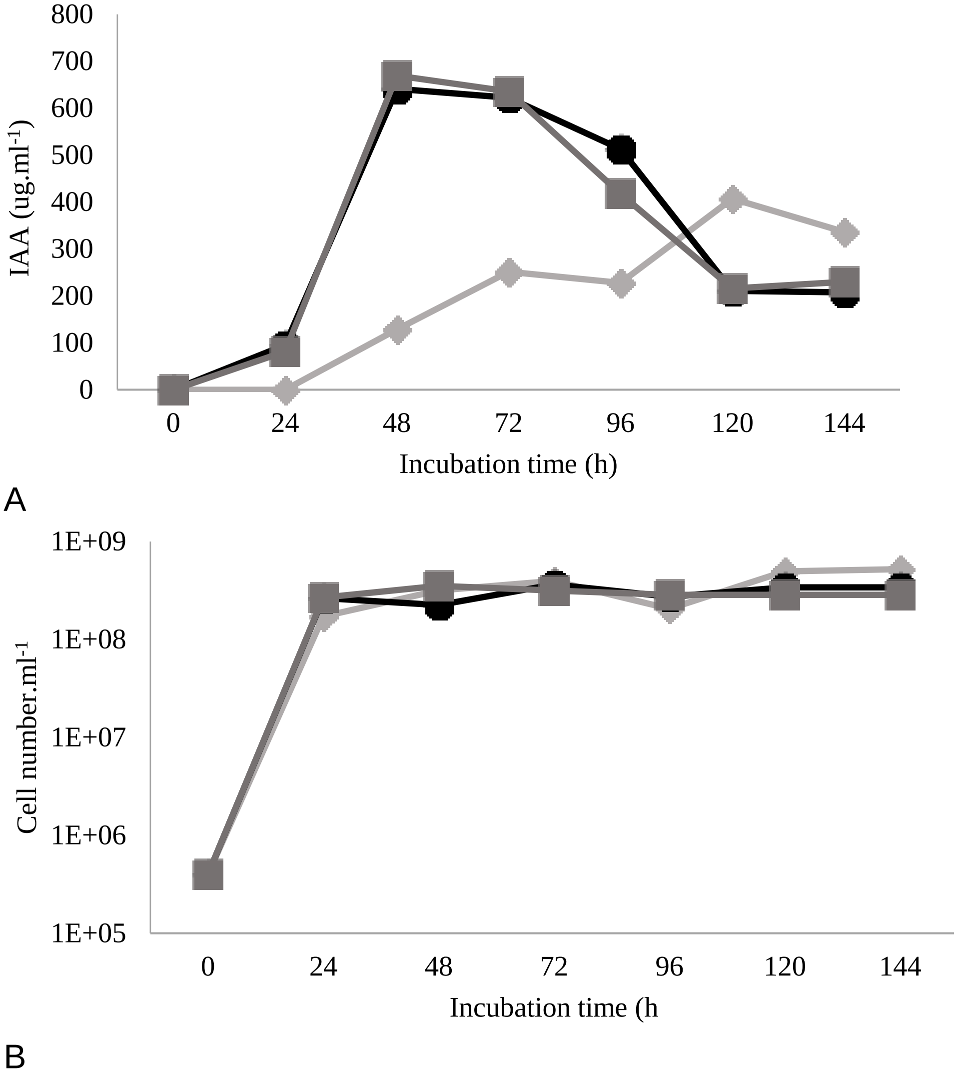

Figure 1. IAA production (A) and cell growth evaluation (B) of yeasts strains 2F32 (R. mucilaginosa) (◆), 5S51 (■) and 5S55 (●) (T. globosa).

Nakayan et al. (2013), who observed that *M. guilliermondii, Rh. mucilaginosa*, and *M. caribbica* were able to produce low rates of IAA, reaching a maximum of 3 μg.ml^{-1} in the absence of tryptophan. Tryptophan is the main precursor for IAA synthesis and in most of the cases, indispensable for the IAA production (Ahmad et al., 2005). It is worth considering that the production of IAA by microorganisms while associated with plants is directly related to the availability of exudates from the roots or leaves (Melo, 1998). The production and concentration of tryptophan may vary in root exudates across various plant species (Patten and Glick, 1996).

The maximum IAA production obtained from both strains (CCA5S51 and CCA5S55) occurred after 48 h of incubation, during the stationary phase of cell growth (Figure 1). After this period, there was a drop in the level of IAA in the medium. This could be explained by the consumption of IAA as a nitrogen source by the yeast. Previous studies have reported that several microorganisms can produce and degrade IAA in the medium (Faure et al., 2009; Scott et al., 2013; Zúñiga et al., 2013). The degradation of IAA, besides being a source of nutrition, must also be a self-protective action of the yeasts, given that excessive IAA in the medium could be toxic to the cells due to acidification of the cytoplasm (Tromas and Perrot-Rechenmann, 2010). A high concentration of IAA could also be toxic to plants (Biswas et al., 2000). As observed in all phytohormones, IAA stimulates plant growth only at low concentrations (Biswas et al*.,* 2000), and is ineffective and toxic at higher

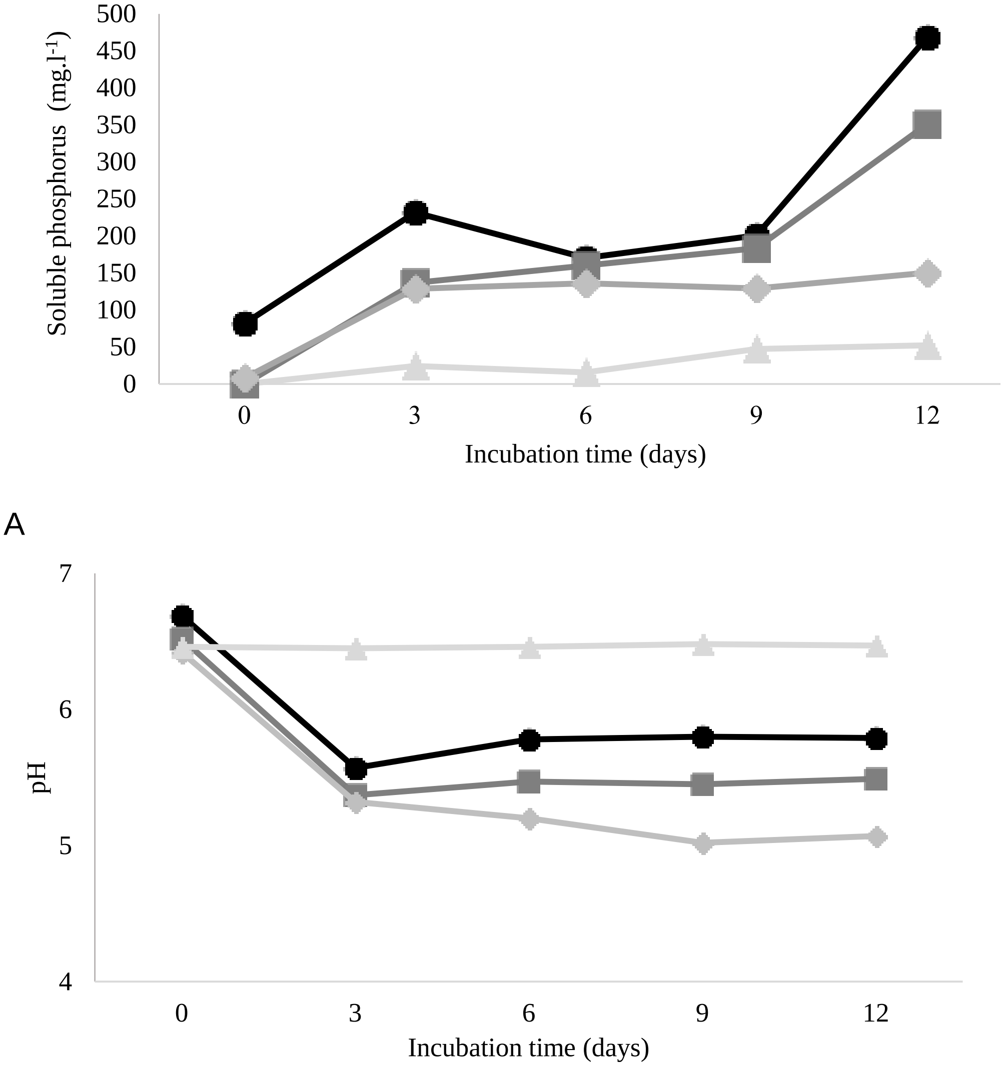

Figure 2. Soluble phosphate (A) and pH (B) observed in the medium of yeast strains cultures 2F32 *(Rh. mucilaginosa) (◆), 5S51 (■) and 5S55 (●) (T. globosa), control (without inoculation) (▲).*

concentrations. Ahmad et al. (2005) inoculated *Sesbania aculeata* and *Vigna radiata* seedlings with *Azotobacter* sp. (IAA producer strain) and found an increase in root elongation at concentrations between 4.4 μg.ml^{-1} and 24.8 μg.ml^{-1} of IAA for the former and 4.4 μg.ml^{-1} to 14.4 μg.ml^{-1} for the latter. Similarly, Barazani and Friedman (1999) reported that rhizobacteria-produced IAA at a concentration around 13.5 μg.ml^{-1} had a deleterious effect on lettuce seedlings. This harmful effect on the plants occurs when the concentration of IAA exceeds the desirable level of stimulation of plant development; IAA in excess has herbicidal action (Fargasova, 1994).

T. globosa (strain 5S55) solubilized 468.2 mg.l^{-1} of phosphate in 288 h (12 days), which was significantly higher than the amount of phosphate solubilized by the other strains. *T. globosa* (strain 5S51) solubilized 350.8 mg.l^{-1} of phosphate, while *M. guilliermondii* (strain 3C98) solubilized 150.4 mg.l^{-1} of phosphate during the same period.

Previous studies in literature attributed the solubilization of minerals to the production of organic acids by the yeast strains. In our study, the flasks with only yeast culture showed a major decrease in the pH of the medium during the incubation, probably due to the release of organic acids. The treatment of yeast culture supplemented with the insoluble phosphorus (tricalcium phosphate) also showed a decline in the pH, but lower probably due to the release of calcium in the culture medium. The flasks with only insoluble phosphorus did not show considerable pH change. The yeast *M. guilliermondii* promoted the highest reduction in pH (Figure 2), reducing it from 6.0 to 2.17 after 72 h of

Table 2. Effect of yeast inoculation, cell concentration, presence and absence of triptophan and glucose on height and dry-weight of tomato seedlings cultivated under greenhouse experimental conditions.

Treatment			**Heigth (cm)**		**Dry-weight (g.plant^{-1})**	
inoculum	**trp***	**glu***	**Shoot**	**Root**	**Shoot**	**Root**
-	-	-	39.37^{b}**	18.25^{b}	0.45^{c}	0.16^{b}
1×10^{8} cells.ml^{-1}	+	+	43.05^{ab}	21.87^{ab}	0.57^{b}	0.39^{ab}
	-	+	42.37^{ab}	22.12^{ab}	0.49^{b}	0.15^{b}
	+	-	45.02^{ab}	23.63^{a}	0.72^{a}	0.22^{b}
	-	-	46.87^{ab}	22.75^{ab}	0.57^{b}	0.21^{b}
3×10^{8} cells.ml^{-1}	+	+	$46,62^{ab}$	23.37^{ab}	0.64^{a}	0.59^{a}
	-	+	40.75^{ab}	21.37^{ab}	0.54^{b}	0.18^{b}
	+	-	44.50^{ab}	20.62^{ab}	0.69^{a}	0.35^{ab}
	-	-	41.75^{ab}	20.08^{ab}	0.63^{b}	0.21^{b}
9×10^{8} cells.ml^{-1}	+	+	41.87^{ab}	25.37^{a}	0.58^{b}	0.35^{ab}
	-	+	41.62^{ab}	20.51^{ab}	0.59^{b}	0.39^{ab}
	+	-	42.07^{ab}	24.12^{a}	0.65^{a}	0.28^{b}
	-	-	49.12^{a}	23.87^{a}	0.72^{a}	0.28^{b}

*Triptophan present (trp+), triptophan absence (trp -), glucose present (glu+), glucose absence (glu-). **Mean followed by same letters within the column did not differ significantly at P<0.1 according to Tukey`s test.

incubation. *T. globosa* (strain 5S55) however, had the highest pH value and maximum phosphate solubilization.

The inoculation of seedlings with the strain 5S55 promotes a significant increase in the dry-weight of shoots, which was seen in all the yeast cell concentrations evaluated. The treatment with an inoculum of the highest yeast cell concentration (9×10^{8} cells.ml^{-1}) affected higher root length in the absence of glucose or in the presence of tryptophan. Thus, tryptophan appears to exert a positive effect in improving the dry-weight of shoots. The treatment with tryptophan, glucose, and 3×10^{8} cells.ml^{-1} improved the dry-weight of the root by 72.8% compared to control (Table 2).

The treatments with glucose and/or tryptophan were significantly superior to those without the treatment. The best results of treatments without glucose and tryptophan were obtained only when the higher yeast cell concentrations were inoculated. This emphasizes the importance of the presence of a source of glucose and/or tryptophan as a stimulus for the yeasts for the establishment of the rhizosphere. This reiterates the importance of the presence of tryptophan in the soil, which is necessary for the promotion of plant growth. Nassar et al. (2005) also observed that the yeast *Williopsis saturnus* (isolate#4), endophytic to maize roots, promoted growth of maize plants, which in the presence of tryptophan was significantly superior to all other treatments and controls in all the comparative parameters. The same study reported that the inoculation of *Rhodotorula glutinis*, a non-IAA producer, in the presence of tryptophan, presented significantly superior results to those from treatments wherein tryptophan was absent.

From the findings of this study, it can be concluded that the yeast species *T. globosa* (strains 5S51 and 5S55) and *R. mucilaginosa* (strain 2F32) produced IAA in the presence of tryptophan, while the species of *T. globosa* (strains 5S51 and 5S55) and *M. guilliermondii* (strain 3C98) solubilized phosphate under *in vitro* conditions. *T. globosa* strain 5S55 showed the best plant growth promotion traits, with maximum IAA production reaching 669 μg.ml^{-1} after 48 h of incubation, and the solubilization of 47% of phosphorus after 12 days of incubation. The development of the tomato seedlings was also improved by inoculation of the 5S55 strain. However, the cell concentration and the presence of glucose and tryptophan significantly influenced the plant growth and must be evaluated in details to attain optimized yields.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Physiological response of colza (*Brassica napus* L.) seeds coated and treated with alternative materials

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The coated seeds have multiple advantages in relation to the common seeds. In addition to the increase in size and uniformity in their shape, treatment products can be incorporated in them helping the germination process. Against the foregoing, the objective of this work was to evaluate the physiological quality of the colza (*Brassica napus* L.) seeds coated with bentonite, gypsum and kaolin and treated with fungicide (carboxin+thiram) and plant extract of black pepper (*Piper nigrum* L.). Colza seeds were encrusted with bentonite, gypsum and kaolin and received or not treatment with fungicide (carboxin + thiram) and aqueous extract of black pepper (*Piper nigrum* L.) which were added together with the cementing mixture (30% of glue of polyvinyl acetate). Germination was evaluated in three periods (4, 7 and 14 days after sowing). The experiment was organized in a completely randomized design and arranged in a factorial scheme (filling materials × treatment products). The means, when necessary, were compared by the Scott-Knott test ($p \leq 0.05$). The seeds coated with gypsum presented the best results for germination and vigor, followed by kaolin and bentonite. The seed coating with gypsum + plant extract presents the same germination and vigor of the seeds without coating.

Key words: Coating, bentonite, physiological response.

INTRODUCTION

The colza/canola (*Brassica napus* L.) is the third most produced oleaginous in the world and it is an annual herbaceous plant belonging to the Brassicaceae family which produces high quality oil-rich grains. This crop is responsible for 15% of the world's edible vegetable oil production, although it is also used in the production of

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biodiesel and animal feed (Tomm et al., 2007). The use of machines in the agriculture is of known importance. They are a responsible part for the expansion of the areas of cultivation and productivity. According to Beltrão and Vieira (2001) one of the obstacles in the mechanical sowing are the seeds with small size, light weight and irregular shape. Colza seeds, according to Angelotti-Mendonça et al. (2016), present such characteristics. The sowing is facilitated when the small seeds are coated, aiming to increase their weight and size, so they flow easily in the seeder (Queiroga and Silva, 2008).

According to Lopes and Nascimento (2012) coating/ pelletizing is a process which consists of depositing dry inert materials of fine granulometry on the surface of the seeds with the assistance of an adhesive material (cementing). This treatment enables to standardize the shape, size and weight of the seeds. Due to this, the distribution of seeds is facilitated, be it manual or mechanized (Nascimento et al., 2009).

In addition, there is the possibility of incorporation of nutrients, growth regulators and other agrochemicals (insecticides and fungicides) during the encrustation / pelletizing process, which may constitute improvements in seed health and seedling establishment (Silva et al., 2002). The use of plants with bioactive potential, in the form of extracts, oils and powders, against various organisms has been increasingly encouraged. Researchers such as Bomtempo (2007), Bong (2010), Cardoso et al. (2005) Abbasi et al. (2010) and Khan et al. (2010) state that piperine, the main compound found in black pepper (*Piper nigrum* L.), has recognized cytotoxic, anti-inflammatory, antipyretic, analgesic, antioxidant, antitumor, antifungal and bactericidal activity.

The process consists basically on applying successive layers of an inert solid material over the seeds in constant movement inside a concrete mixer, alternating the application of the filling material with the spraying of a water-soluble cementing material (Silva, 1997; Silva and Nakagawa, 1998). It is necessary to attend to the fact that the main objective of the seed coating technique is to optimize the behavior of the seeds, both from the physiological and economic point of view. Consequently, the choice of the coating materials is important so they do not affect negatively the seeds vigor and germination (Oliveira et al., 2003).

According to Baudet and Peres (2004) the specific details of this methodology are not informed as they are considered trade secrets, and thereby only the general description of processes and inputs used are available. Thus, it is essential to study the behavior of the seeds through the use of different materials for the filling, as well as the treatment products through tests of germination and vigor. In light of the foregoing, the objective of this work was to study the performance of the colza seeds (*B. napus* L.) coated with bentonite, gypsum and kaolin and treated with fungicide and plant extract of black pepper (*Piper nigrum* L.) as for the germination and vigor.

MATERIAL AND METHODS

Plant material

The seeds were purchased from the local market of the city of Campina Grande, Paraíba, Brazil. After acquisition, the seeds were taken to the Laboratory for cleaning to remove all strange material that came along with the seeds. The experiment was conducted at the Laboratório de Armazenamento e Processamento de Produtos Agrícolas (LAPPA), in the Universidade Federal de Campina Grande, Campina Grande Campus, Paraíba, Brazil.

Preparation of the plant extract

The fruits of black pepper (*P. nigrum* L.) were purchased at the central fair of the city of Campina Grande, Paraíba, Brazil. The aqueous extract was obtained from the powder of the fruits, which were weighted, moistened with distilled water and kept in an infusion for 72 h, at ambient temperature of 24.0 ± 4.0°C, in the absence of light and with daily shaking for five minutes. The amount of powder used corresponded to 20% of the volume of water used. Posteriorly, the solutions were filtered on filter paper and the extract was stored in an amber glass container with capacity for 0.5 L (Almeida et al., 2004).

Materials and process of seed coating

Three filling materials were used: (1) bentonite, (2) gypsum and (3) kaolin. As cementing material, the PVA (polyvinyl acetate) glue was used at the percentage of 30% (Melo et al., 2016), for each filling material. As treatment products, the aqueous extract of black pepper (*P. nigrum* L.) and a fungicide (carboxin+thiram) were used, corresponding to 50% of the mixture (Table 1).The seed coating process occurred by the alternating application of cementing material and filling material in a machine developed for this purpose. This process was repeated until all the material destined to the process has been totally used.

Germination test

The germination test was conducted with four sub-samples of 50 seeds; they were sown in plastic trays with vermiculite, moistened with distilled water with volume corresponding to 60% of the holding capacity. These were kept in ambient conditions of temperature, relative air humidity and photoperiod. The germination percentages were recorded after 4 and 7 days form beginning of the treatments (Brasil, 2009). A third germination count was also performed at 14 days after sowing to evaluate whether there was reduction or delay in germination.

Dry matter of the aerial part

To determine the dry matter of the aerial part, the seedlings, at the second count, were cut at the height of the substrate surface and put in *kraft* paper bags. After that, they were subjected to drying in an oven with forced air circulation, at a temperature of 65°C until reaching constant weight. Thereon, the plant material was weighted in a precision digital scale and the data were expressed in milligrams (mg).

Table 1. Treatments used in the encrustation process.

Treatments	PVA glue	Fungicide	Vegetable extract
Seeds without encrustation	-	-	-
Bentonite Gypsum Kaolin	Cementing material (30% PVA glue + 70% distilled water)	Cementing material (30% PVA glue + 50% fungicide + 20% distilled water)	Cementing material (30% PVA glue + 50% vegetable extract + 20% distilled water)

Experimental design and statistical analysis

The experiment was organized in a completely randomized design and arranged in a 4 × 3 factorial scheme (filling materials x treatment products). Each treatment was repeated four times. The data were submitted to Analysis of Variance ($P \leq 0.05$) and the means, when necessary, were compared by the Scott-Knott test ($P \leq 0.05$).

RESULTS

Table 2 organized the values of the mean squares for the first germination count (FCG), germination (G), third germination count (TCG) and dry matter of the aerial part (DMAP) of the seedlings from the colza seeds coated with bentonite, gypsum and kaolin and treated with fungicide and plant extract of black pepper. It is verified for all the studied variables a highly significant effect for the isolated or interacting factors, revealing statistical differences between the treatments. Comparing the treatment products within each filling material, the mean germination for the control was of 57.50%. For the bentonite, the highest germination was observed when the glue was used, followed by the plant extract and the fungicide, these presenting statistical difference between them. For gypsum and kaolin, the highest germinations were observed when the plant extract was used. On the other hand, the lower germinations, for these two materials, were observed when the fungicide was used. For these materials, the glue presented intermediate behavior (Table 3).

When comparing the filling materials within each treatment product, it is verified that for the glue the highest germinations occurred in the control and when the gypsum was used, being statistically equal to each other, and different from the germination observed when using kaolin and bentonite. When the fungicide was used it can be verified that all the treatments were statistically different among them, with higher germination in the control and lower in the bentonite. The kaolin and the gypsum presented intermediate behaviors (Table 3).

When the plant extract of black pepper was used, it was verified that the gypsum and the control presented the highest germinations. On the other hand, the lowest germination was observed when the seeds were coated with the bentonite. Seeds that were coated with kaolin showed germinations with intermediate behavior in relation to the other treatments (Table 3). When comparing the filling materials within each treatment product, it is verified that the highest germination was verified in the control, differing statistically from the gypsum. On the other hand, the lowest germinations were observed when bentonite and kaolin were used, not differing statistically from each other. In relation to the fungicide, it is verified that the highest germination was verified in the control and the lowest one when the bentonite was used. The coating of colza seeds with gypsum and kaolin provided seeds with intermediate germinations in relation to the other materials (Table 4).

When using the black pepper plant extract, it was verified that there was no statistical difference between the germination of the seeds of the control and those coated with gypsum. These germinations were statistically different from those observed when the seeds were coated with kaolin and when bentonite was used (Table 4).

It can be verified that in the control the mean germination was of 71.50%. For the bentonite, it was observed that the glue provided a higher germination, differing from the plant extract of black pepper and the fungicide, which presented the lowest germination among the treatment products for this filling material. As for the gypsum and kaolin, the greatest germinations occurred when the black pepper plant extract was used, differing from the glue. The fungicide, in these two materials, provided the lowest germinations (Table 4). When comparing the treatment products within each filling material, it was verified that in the control the mean germination was of 72.0%. In relation to the bentonite, it is observed that the glue and the fungicide provided the highest germinations, which were significantly different from the germination observed when the plant extract was used. Regarding the gypsum, it is verified that the three products differed from each other. When using the plant extract the germination was the highest among the three products, followed by the germination observed when the glue and the fungicide were used. As for the kaolin, the highest germination was verified when the plant extract was used, differing from the glue and the fungicide, being these statistically equal (Table 5).

On the other hand, when comparing the filling materials within each treatment product, it can be verified that for the glue the highest germination occurred in the control, which differed from the germination when the gypsum

Table 2. Mean squares referring to the first count (FCG), second count (G), third count (TCG) and dry matter of the aerial part (DMAP) of the seedlings from colza (*B. napus* L.) seeds coated with different filling materials (FM) and percentages of cementing material (PC).

Source of variation	Mean squares				
	FD	FCG	SCG	TCG	DMAP
FM	3	2496.66**	1994.05**	772.08**	10257.63**
PC	2	1723.58**	1540.18**	121.33**	6543.75**
FM x PC	6	307.91**	292.15**	110.00**	1015.97**
Error	36	15.38	13.62	12.91	107.63

**Significant at 1%.

Table 3. Means of the first germination count (%) of colza (*B. napus* L.) seeds coated with bentonite, gypsum and kaolin and treated with fungicide and plant extract of black pepper.

Filling materials	Treatment products		
	PVA glue	Fungicide	Plant extract
Control	57.50±0.83^{aA}	57.50±0.83^{aA}	57.50±0.83^{aA}
Bentonite	34.00±2.49^{cA}	8.00±1.58^{dC}	26.00±2.45^{cB}
Gypsum	54.00±1.87^{aB}	24.00±2.12^{cC}	60.00±0.71^{aA}
Kaolin	44.50±1.92^{bB}	32.00±1.87^{bC}	52.50±1.48^{bA}

*Means followed by the same lowercase letter in the column and upper case in the row do not differ from each other by the Scott-Knott test (P ≤ 0.05). CV% = 9.27.

Table 4. Means of the second germination count of seeds (%) of colza (*B. napus* L.) coated with bentonite, gypsum and kaolin and treated with fungicide and plant extract of black pepper.

Filllingmaterials	Treatment products		
	PVA glue	Fungicide	Plant extract
Control	71.50±1.09^{aA}	71.50±1.09^{aA}	71.50±1.09^{aA}
Bentonite	54.50±2.28^{cA}	23.00±2.50^{cC}	44.00±1.22^{cB}
Gypsum	63.50±1.48^{bB}	39.50±2.17^{bC}	70.75±0.82^{aA}
Kaolin	53.00±1.50^{cB}	42.75±0.82^{bC}	60.50±1.92^{bA}

*Means followed by the same lowercase letter in the column and upper case in the row do not differ from each other by the Scott-Knott test (P ≤ 0.05). CV% = 6.65.

was used, and also differed from the bentonite and kaolin, which did not presented statistical difference between them (Table 5).

Regarding the fungicide, the highest germination was verified in the control, differing from the other materials. In the case of the black pepper plant extract, it is verified that the germination when the gypsum was used did not differ from the control. However, they differed from the germination registered when the kaolin was used, and also when the bentonite was used, with the lowest germination among the materials (Table 5).

In Table 6 are the mean values for the dry matter of the aerial part of seedlings from colza seeds coated with bentonite, gypsum and kaolin and treated with fungicide and black pepper plant extract. When comparing the filling materials within each treatment product, it is verified that for the PVA glue the highest value for dry matter was observed in the control. On the other hand, the lowest values for dry matter of the aerial part were registered when the seeds were coated with bentonite and kaolin. However, the coating of seeds with gypsum showed intermediate values for the dry matter of the aerial part.

Comparing the filling materials within the fungicide, it can be verified that the highest value of dry matter was found in the control, differing statistically from the values of dry matter for seeds coated with gypsum and kaolin, which were statistically equal but different from the dry matter of the aerial part observed when the seeds were coated with bentonite. As for the plant extract, it can be

Table 5. Means of the third germination count (%) of colza (*B. napus* L.) seeds coated with bentonite, gypsum and kaolin and treated with fungicide and black pepper plant extract.

Filling materials	Treatment products		
	PVA glue	**Fungicide**	**Plant extract**
Control	72.00±0.71^{aA}	72.00±0.71^{aA}	72.00±0.71^{aA}
Bentonite	59.00±1.66^{cA}	55.50±1.30^{bA}	49.50±0.43^{cB}
Gypsum	66.00±1.87^{bB}	56.50±2.86^{bC}	72.50±1.09^{aA}
Kaolin	56.50±1.92c^{B}	51.50±1.09^{bB}	61.50±2.28^{bA}

*Means followed by the same lowercase letter in the column and upper case in the row do not differ from each other by the Scott-Knott test (P ≤ 0.05). CV% = 5.79.

Table 6. Means of the dry matter of the aerial part (mg) of seedlings from colza (*B. napus* L.) seeds coated with bentonite, gypsum and kaolin and treated with fungicide and plant extract of black pepper.

Filling materials	Treatment products		
	PVA glue	**Fungicide**	**Plant extract**
Control	140.00±3.54^{aA}	140.00±3.54^{aA}	140.00±3.54^{aA}
Bentonite	92.50±2.17^{cA}	37.50±2.17^{cB}	80.00±3.54^{cA}
Gypsum	120.00±6.12^{bA}	72.50±4.15^{bB}	132.50±9.60^{aA}
Kaolin	95.00±4.33c^{B}	70.00±3.54^{bC}	117.50±2.17^{bA}

*Means followed by the same lowercase letter in the column and upper case in the row do not differ from each other by the Scott-Knott test (P ≤ 0.05). CV% = 10.06.

observed that the highest values of dry matter were registered in the control and when the seeds were coated with gypsum, not differing statistically between each other. On the other hand, they differed statistically when the kaolin and the bentonite were used, which differed statistically from each other (Table 6).

When comparing the treatment products within each filling material, it is verified that in the control the mean value for dry matter of the aerial part was 140.0 mg. For bentonite and gypsum, the highest values of dry matter of the aerial part were registered when only the glue was used and in combination with the plant extract of black pepper. For the kaolin, the highest value of dry matter of the aerial part was registered when using the black pepper plant extract, differing statistically from the one observed when using the glue and the fungicide, which was the lowest dry matter value among the treatment products (Table 6).

DISCUSSION

The reductions of germination and vigor of the coated seeds were also verified by Oliveira et al. (2003), who reported that some materials, among them the fungicide, used in the coating of the seeds, as well as their dosage, may cause immediate phytotoxic effects in the germination or reduce the physiological quality of the seeds. Franzin et al. (2004) also verified reductions in the germination and vigor of pelleted lettuce (*Lactuca sativa* L.) seeds in relation to non-pelleted seeds, and they also suggested that these seeds should be evaluated by means of specific tests. Ferreira et al. (2015) while evaluating the physiological quality of six batches of coated seeds of hybrid brachiaria, cv. Mulato II, verified that the coated seeds presented a significant reduction in relation to the bare seeds. These authors suggest that such reduction was due to the physical barrier created by the materials used in the coating process. In this respect, Sachs et al. (1981), reported that most of the coating materials used hinder the penetration of oxygen in the seed. Giménez-Sampaio and Sampaio (1994), support the opinions above by stating that the obtained results for coated seeds and bare seeds cannot be interpreted in the same way, since the tests used to evaluate the germination capacity and vigor of the coated seeds are the same.

In general, the gypsum stood out in relation to the germination and vigor of the seeds. Despite the germination observed with the gypsum, alone, it is not a decisive parameter for the choice of the coating material. It must have a capacity to increase the diameter of the seed in a satisfactory way, and in addition to other characteristics, it must have resistance sufficient for the use in the mechanized planting. The bentonite is a clay stone that can be calcium or sodium with high capacity of water absorption and low permeability and, when moistened, makes a viscous gel that expands it by increasing its volume (Tonnesen et al., 2012). The high viscosity attributed to the bentonite causes a resistance to flow (Menezes et al., 2009), which explains a decrease in the germination of the colza seeds coated with this material. Despite the lower germination of seeds covered with the bentonite in relation to the other materials, it was promising in the coating process of colza seeds, resulting in a reduction of 20% of the germination in relation to the control, counted in the third count. Also, the bentonite presents the characteristics of increased size, weight and greater resistance required for a good coating covering. The germination result suggests testing other cementing materials and/or percentages of them, and a finishing polymer that can promote a decrease of this difference of germination with the control.

Conclusions

In view of the above, it can be concluded that the germination and the vigor are affected by coating. Seeds coated with gypsum present the best results for germination and vigor, followed by kaolin and bentonite. The coating of the seeds with gypsum + plant extract presents the same germination and vigor of seeds without coating. The fungicide damages the germination and vigor of the colza seeds regardless of the coating material used.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Interactive effects of phosphorus and water stress on plant development and yield resilience in common beans (*Phaseolus vulgaris* L.)

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An experiment was conducted to determine the effects of increasing soil phosphorus under water stress conditions on yield and plant development in bean (*Phaseolus vulgaris* L.) genotypes with contrasting drought susceptibility tolerances. A split-split plot design with genotypes (main plot), two water regimes (split plot) and three phosphorus rates (split-split plot) was used. Water regimes were imposed by irrigating at 50% ET (water stress) and 100% ET (no stress). Phosphorus (P_2O_5) was applied at planting at the following rates- 0, 40 and 70 kg P ha^{-1}. The two bean lines used were Gadra (high drought susceptibility) and KE- 3 (low drought susceptibility). Water stress significantly reduced plant height, shoot biomass, pod length, seeds per pod, pods per plant, days to maturity and grain yield in both genotypes. Phosphorus significantly increased grain yields mainly through increased number of pods per plant and 100-seed weight. Higher increases were observed in Gadra where moderate P application increased yield from about 250 to 1,000 kg ha^{-1} and high P increased yield to 1,600 kg ha^{-1}. The results suggested that high P foraging and utilisation efficiency were inversely related.

Key words: Biomass, soil nutrients, phytotoxicity, yield, yield components.

INTRODUCTION

Common bean (*Phaseolus vulgaris* L.) is the grain legume with the highest volume of direct human consumption in the world and is the most important legume in Eastern and Southern Africa cultivated over an area of about 4 million ha (Beebe et al., 2014). Worldwide per capita consumption is high but varies according to region, being as high as 60 kg in East Africa and about 4 - 17 kg in Latin America (Beebe et al., 2013; Broughton et al*.,* 2003). Generally, productivity is low, with a global average yield of 715 kg ha^{-1} against a potential yield of 1,500 to 3,000 kg ha^{-1}. In Africa, the average yield is about 500 kg ha^{-1} which is well below the global potential yield (Namugwanya et al., 2014). The reasons for this low productivity are due to both biotic and abiotic factors. Beans are particularly sensitive to abiotic factors rendering them unable to reach their productive potential

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(Assefa et al., 2015; Velho et al., 2018). Drought and soil infertility, especially phosphorus deficiency, are primary constraints to crop production in many developing countries, affecting over 80% of bean production regions in the world, and they frequently co-occur throughout the tropics (Suriyagoda et al*.,* 2014). In Sub Saharan Africa drought is the most important risk, potentially affecting as much as one- third of the production area (Beebe et al*.,* 2014).

Water is essential for many plant processes, primarily acting as a solvent for different metabolites, providing means for nutrient and solute transport and also involved in plant temperature regulation (Baker, 1984). Soil water deficits severely retard plant development, reducing yield (Mataa et al., 1998). It is reported that 60% of common bean production is located in drought prone areas and the increasing competition with major crops continues to push common beans into marginal lands with increased risk of drought stress (Mukeshimana et al*.,* 2014). The amount of fresh water is finite and water has multiple and competing uses including direct human, industrial and agriculture uses. Efficient utilization of fresh water in agriculture can greatly contribute to water conservation.

Besides nitrogen, phosphorus is the most limiting nutrient for plant growth in arable soils (Suriyagoda et al., 2010). Phosphorus in plants is important for variety of processes particularly in maintenance of energy-metabolic systems, enhanced root development and root hydraulic conductance (Jin et al*.,* 2014). Plants have evolved different strategies for P acquisition and use in limiting environments (Ho et al*.,* 2005; Vance et al*.,* 2003). These include increased mycorrhizal symbioses, decreased growth rate, remobilization of internal inorganic phosphate, modification of carbon metabolism bypassing P-requiring steps, increased synthesis and secretion of phosphatase, exudation of organic acids, and enhanced expression of P transporters (Raghothama, 1999; Plaxton, 2004; Vance et al., 2003). In response to low P, bean genotypes can either exhibit high P acquisition efficiency (PAE) and/or P utilization efficiency (PUE) (Atemkeng et al*.,* 2011; Cichy et al*.,* 2009; Vandamme et al*.,* 2016). PUE is the efficiency at which P taken up is converted to biomass whereas PAE is the ability of a plant to mobilize and absorb more P from the fertile soil layers (Atemkeng et al*.,* 2011). Typically, PAE includes modifications in root architecture to increase capacity of the plant roots to explore more soil in the upper soil layers which have higher P content. Since P is relatively immobile in the soil, it is important that the ability of plants to explore greater soil volume is enhanced if they are to access more of the nutrient. To achieve this, plants develop traits that include increased root development, higher root: shoot ratios, finer roots and longer root hairs (Suriyagoda et al*.,* 2010). However these adaptations are usually inadequate to meet the minimum phosphorus requirements for normal plant development and the application of external phosphorus is still a primary requirement for crop cultivation (Vance et al*.,* 2003). Soil water deficits further restrict phosphorus movement in the soil and this situation is projected to worsen with climate change. Although low soil P and soil water deficits are recognized as primary factors limiting bean productivity, most research has tended to focus on each of these factors separately despite them tending to occur as together (Namugwanya et al*.,* 2014; Suriyagoda et al*.,* 2014). It is known that soil related constraints can seriously limit the potential expression of drought resistance and it is therefore important to address multiple stresses (Beebe et al*.,* 2014; Suriyagoda et al*.,* 2014).

The objective of the study was to evaluate the effect of varying soil phosphorus content and water stress on yield and plant development in common bean genotypes with differing drought susceptibility tolerances.

MATERIALS AND METHODS

Experimental site

The study was carried out at National Irrigation Research Station (S 15° 046' 43" and E 27° 055' 14", in Mazabuka, Southern Zambia from September to December 2012. The soils belong to the Nakambala series and according to USDA soil taxonomy are classified as *typic kanhaplustalf*.

Chemical analysis

Total nitrogen (N) was determined by the Kjeldahl digestion-distillation method (Bremner and Mulvancy, 1982). The determination of available phosphorus (Bray 1 Method) was conducted according to Bray and Kurtz (1945). Soil pH ($CaCl_2$) was determined using a digital electronic pH meter. Soil organic matter was determined according to the Walkley and Black (1934). Soil analyses showed that total nitrogen was 0.09% and available phosphorus was 8.5 mg kg $^{-1}$.

Plant materials

Two bean genotypes (Gadra and KE-3) genotypes were obtained from the Seed Control Certification Institute, Zambia. Selection of the genotypes was based on differences in drought susceptibility index with Gadra having high drought susceptibility and KE-3 low drought susceptibility (Kalima, 2013).

Field layout and crop management

There was 1.0 m space separating each treatment of phosphorus and variety within sub plots. There were 5 m buffer rows surrounding the field and a 2-m buffer zone separating the different irrigation regime portions of the field. The entire field received 40 kg N ha^{-1} as urea and 40 kg K ha^{-1}. For the three P treatments; low P- no P was added (0 kg P ha^{-1}), medium P- 40 kg P ha^{-1} was applied, and the high phosphorus rate field received 70 kg P ha^{-1}. The phosphorus fertilizer was applied as basal dressing of mono ammonium phosphate at planting.

Planting was done on the 6th of September, 2012. Standard bean management practices were used as recommended in Zambia. The field was kept weed free by regular weeding and monocrotophos was applied to control the bean fly, *(Ophiomyia phaseoli)* (Tryon) (Diptera: Agromyzidae). Commercial termiticide and nematicides were applied at planting to prevent termite and nematode attack, respectively.

Water stress application

For irrigation, the field was divided into blocks of 4.0 m × 7.4 m per replicate, with each block receiving either, 100% irrigation (no stress) or irrigation at 50% evapotranspiration (stress). Water stress was imposed at pre-flowering stage (V8), and discontinued when the plants were in their late reproductive stage (R8) (Kandel, 2010). Plants were harvested after physiological maturity in second week of December before seed shattering.

Measured parameters

The following parameters: (1) number of pods per plant; (2) number of grains per pod, were measured after harvest of the crop. (3) number of pods per plant; (4) number of days from emergence to physiological maturity of the crop [this was done by visual observation on the number of days to the time when the leaves start losing the green colour (Kandel, 2010)]. Plants were considered physiologically mature when at least 80% of the pods turned yellow (Kandel, 2010). (5) length of the vegetative phase (days to flowering); (6) plant height. Plant height was measured at physiological maturity by determining the distance from the ground level to the tip of the shoot apex; (7) pod length (cm) at physiological maturity of the crop. Pod length was expressed as the average of about 10 pods per plant. 8) 100-seed weight. The harvested seeds were air-dried in the shade for about 2 weeks and 100-seed samples from each plot were measured. 9) aboveground biomass accumulation was obtained by cutting the plants at ground level and drying the biomass in a forced drought oven at 60°C for 72 h.

Experimental design and data analysis

A split-split plot design, with genotypes (main plot), water regimes (split plot), phosphorus levels (split-split plot) replicated three times was used (Sokal and Rolfe, 1981). Data was analysed using GenStat 16 Software. Data was subjected to analysis of variance and where significant treatments effects ($p \leq 0.05$) were discerned, means were separated using least significant difference (LSD).

RESULTS

Table 1 summarizes the single, multiple and interactive effects of the treatment factors. All the three factors exerted significant effects on most of the measured parameters. There were similarities and differences in the way the two genotypes responded to water supply and phosphorus supply. Vegetative parameters such as plant height, days to maturity, and canopy biomass were affected by the water stress and phosphorus treatments. The water and phosphorus parameters affected yield and all yield components. Except for days to maturity and number of seeds per pod, very highly significant interactions between water stress and phosphorus levels were observed.

Vegetative parameters

Plant height

Effects of genotype, water stress and phosphorus on vegetative parameters are presented in Table 2. The genotype KE-3 had taller plants compared to Gadra. Overall, water stress significantly reduced plant height. In terms of effects of P on plant height, additional P increased plant height ($p \leq 0.001$). There were highly significant interactions between genotype and water regime (Table 1). The reduction in plant height due to water stress was higher in Gadra than KE-3. There were significant differences in response of the two genotypes to P; in both genotypes, P increased plant height but for Gadra, the lower P (40 kg ha^{-1}) rate caused about 25% height increase whereas the higher rate (70 kg ha^{-1}) reduced plant height, possibly indicating phyto toxicity. KE-3 had a consistent increase in plant height as more P was added; the 40 kg ha^{-1} treatment caused a 35% increase and the 70 kg P had an 80% plant height increase. There was a significant interaction between water regime and soil P; adding P increased plant height for both normally irrigated and water stressed treatments but the response was higher under the normally irrigated plots. Compared to the no- P addition, increases in plant height for the 40 and 70 kg ha^{-1} P treatments were 18 and 15% respectively under water stress and 19 and 68% respectively for the normally irrigated treatments.

Figure 1A showed the interaction of all the 3 factors- genotype, water regime and P levels on plant height. There was a highly significant interaction between genotype, water regime and phosphate application ($p \leq 0.001$) (Table 1). Applying P under normal irrigation increased plant height consistently in KE 3, but under water stress, additional P (70 kg ha^{-1} P) caused reduction in plant height in Gadra. For Gadra, addition of 70 kg ha^{-1} of P caused about 10% height reduction compared to the plant height of the 40 kg ha^{-1} P treatment.

Days to full maturity

The effects of treatments on days to full maturity (DFM) are given in Table 2. Gadra had a significantly shorter DFM phase compared to KE-3. Water stress had a highly significant ($p \leq 0.001$) effect on the days to full maturity. Overall, when the plants were subjected to water stress the DFM decreased by almost 15%. On average, addition of P had a highly significant effect ($p \leq 0.001$) on DFM; it increased the DFM from about 63 to 69 days under the 40 kg ha^{-1} P and to 74 for the 70 kg ha^{-1} treatment respectively. The increase was significantly larger under the 70 kg ha^{-1} P treatment compared to the 40 kg ha^{-1} P rate. Significant genotype and water stress interactions were observed (Table 2). Generally, water stress reduced the DFM, and the decrease was higher in Gadra (21%) compared to KE (10%). Highly significant ($p \leq 0.001$)

Table 1. Summary ANOVA table showing significance of different sources of variability.

Source	Plant height	Days to maturity	Canopy biomass	Seeds per pod	Pods per plant	Pod length	100- seed weight	Yield
Water (W)	***	**	**	*	***	**	ns	**
Phosphorus (P)	***	***	**	***	**	**	***	***
Genotype (G)	**	***	***	ns	***	ns	***	***
W × P	***	***	***	*	***	***	ns	***
W × G	***	***	***	ns	***	***	***	***
P × G	***	***	***	ns	***	***	***	***
G × W × P	***	ns	***	ns	***	***	**	***

Factor significance; *** highly significant ($p \leq 0.001$); **very significant ($p \leq 0.01$); * significant ($p \leq 0.05$) and ns- non significant.

genotype and phosphorus interactions were observed (Table 1). Addition of P increased the DFM with KE-3 having a larger increase compared to Gadra. Water stress and phosphorus interacted in responses to DFM ($p \leq 0.003$). The increase in DFM due to P under water stress conditions was less compared to normally irrigated treatments.

Significant genotype, water stress and phosphorus interactions in DFM were observed (Figure 1B, Table 1). DFM increased with increase in P addition, but these increases occurred where irrigation was normal. Water stress reduced DFM when accompanied with P addition and reduced DFM in Gadra at the 70 kg ha^{-1} P level. However, KE-3 showed consistent increase in DFM with more P. Overall, the highest DFM occurred in KE-3 under normal irrigation and 70 kg ha^{-1} P application.

Effect of water supply and phosphorus application on biomass accumulation

The effects of water and phosphorus application on biomass for the two genotypes are presented in Table 2 and Figure 1C). The effects of water stress and P on biomass were similar to those observed for plant height and DFM. However, canopy biomass was more sensitive to water stress and changes in soil P. Water stress had a significant effect ($p \leq 0.005$) on biomass accumulation. On average water stress reduced canopy biomass by about 25%. In terms of effects of P application, plant biomass increased with P addition, with higher responses being detected for the 40 kg ha^{-1} P application compared to the 70 kg ha^{-1} treatment.

Highly significant ($p \leq 0.001$) genotype and water stress interactions were observed. Biomass accumulation in Gadra was more sensitive to water stress as it experienced a 41% biomass reduction under water stress compared to KE-3 which had a 14% decrease. Highly significant genotype and P interactions were observed. Both genotypes responded to P addition by increasing biomass but the response patterns were different. KE-3 had a consistent increase in biomass with P addition. On the other hand, Gadra was less tolerant of high soil phosphorus. The application of 70 kg ha^{-1} P resulted in a significant reduction in canopy biomass compared to both the 40 kg ha^{-1} P and the treatment where no P was added. Significant water and P interactions were evident (Table 2). Under water stress conditions, although both P additions caused significant increases in canopy biomass, the lower P (40 kg ha^{-1}) treatment showed a larger increase in biomass (51%) compared to 12% increase for the 70 kg ha^{-1} P treatment. Under normally irrigated treatments the increases for the 40 and 70 kg ha^{-1} treatments were 21 and 114%, respectively. This showed larger increases for the normally irrigated treatments and possibly indicating that water deficit reduced the plant's ability to use additional P to increase its biomass. P addition only caused higher canopy biomass production if there was no water stress.

Reproductive parameters

Yield

Table 3 shows the effects of genotype, soil water stress and phosphorus content on seed yield. As noted earlier with the vegetative parameters, water stress caused highly significant reductions

Table 2. Single and 2 way interactive effects of genotype, water regime and phosphorus levels on vegetative development of common beans (*P. vulgaris* L.).

Factor		**Parameter**			
			Plant height (cm)	**Days to maturity (days)**	**Canopy biomass(g/plant)**
Genotype (G)					
Gadra		G1	25.7	59.9	27.2
KE 3		G2	54.4	76.1	45.4
	Lsd Genotype (G)		0.73	0.70	0.002
Water regime (W)					
Stressed		W1	37.1	62.6	30.9
Normal		W_2	43.1	73.4	41.7
	Lsd Water regime (W)		1.81	2.52	0.005
Phosphorus (P)					
		P_0	30.8	61.7	28.8
		P_1	40.5	68.5	41.2
		P_2	49.1	73.9	42.9
	Lsd Phosphorus (P)		1.21	0.59	1.04
G × W		$G_1 W_1$	23.7	52.9	20.2
		$G_2 W_1$	50.6	72.3	41.6
		$G_1 W_2$	27.8	67.0	34.2
		$G_2 W_2$	58.3	79.9	49.2
	Lsd G x W		1.45	2.01	1.66
G × P		$G_1 P_0$	22.3	52.8	17.2
		G_1P_1	28.0	62.2	36.7
		$G_1 P_2$	27.0	64.8	27.8
		G_2P_0	39.2	70.5	30.5
		G_2P_1	53.0	74.8	45.8
		$G_2 P_2$	71.2	83.0	60.0
	Lsd G × P		1.40	0.98	1.32
W × P		$W_1 P_0$	30.2	60.2	22.5
		$W_1 P_1$	35.7	62.8	36.3
		$W_1 P_2$	45.5	64.8	34.0
		$W_2 P_0$	31.3	63.2	25.2
		$W_2 P_1$	25.3	74.2	46.2
		$W_2 P_2$	52 7	83.0	53.8
	Lsd W × P		1.69	2.05	1.66
Factor significance		**Genotype (G)**	0.005	≤ 0.001	0.002
		Water regime (W)	≤ 0.001	0.003	0.005
		Phosphorus (P)	≤ 0.001	≤ 0.001	≤ 0.001
		G × W	≤ 0.001	≤ 0.001	≤ 0.001
		W × P	≤ 0.001	≤ 0.001	≤ 0.001
		G × P	≤ 0.001	≤ 0.001	≤ 0.001

on reproductive parameters ($p \leq 0.003$). In terms of genotype, KE-3 had a higher yield compared to Gadra. Addition of phosphorus to the soil increased yield significantly by about 160 and 230% under the 40 and 70 kg ha^{-1} P treatments, respectively. With respect to yield, water stress and genotype showed significant

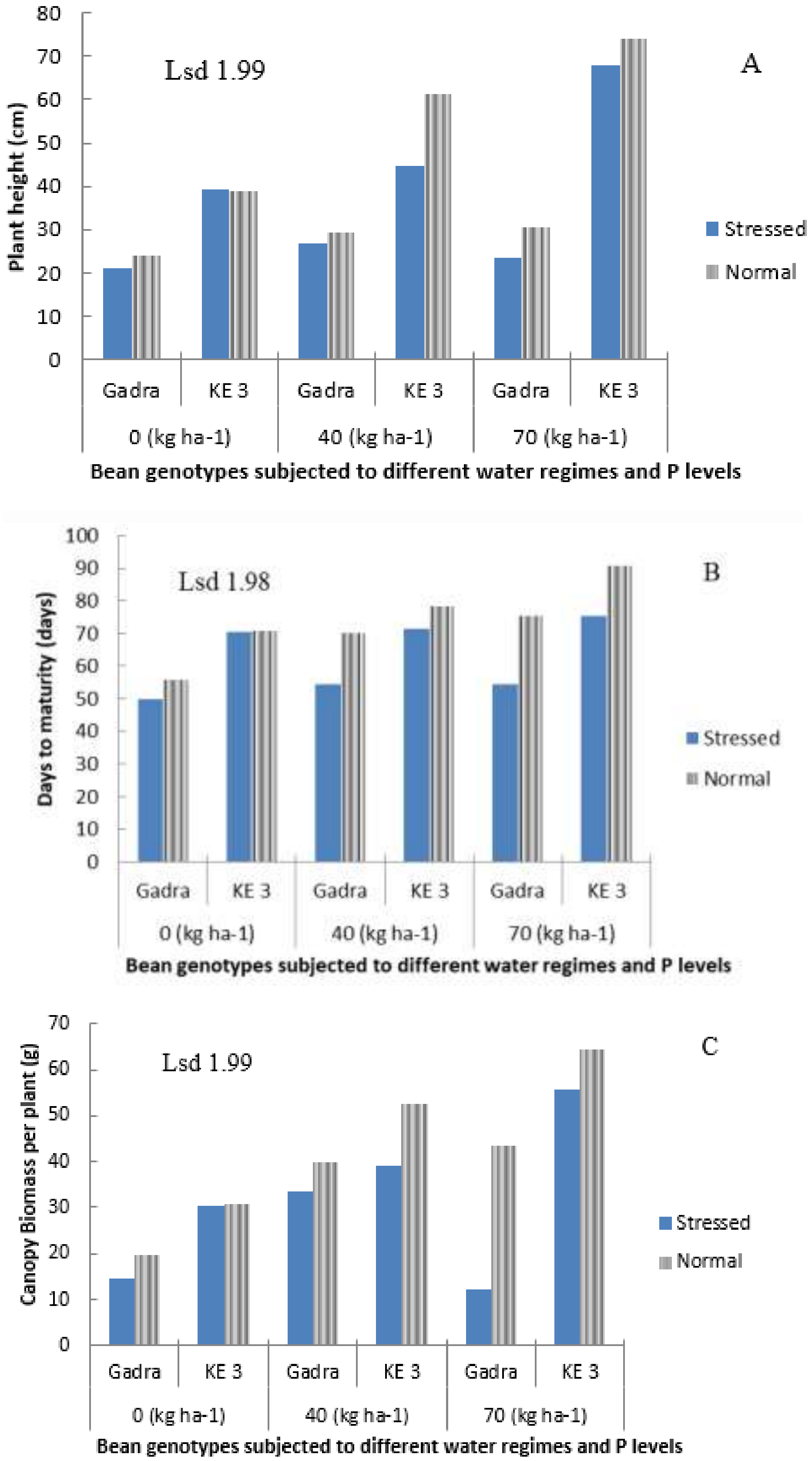

Figure 1. Three way interactive effects of genotype, water regime and phosphorus levels on vegetative development of common beans (*P. vulgaris* L.).

Table 3. Single and 2 way interactive effects of genotype, water regime and phosphorus levels on reproductive parameters in common beans (*P. vulgaris* L.).

Factor			**Parameter**		
			Yield	**Pods/Plant**	**Pod length**
Genotype (G)					
Gadra		G1	894.2	20.8	7.9
KE 3		G2	1410.6	16.9	7.6
	Lsd Genotype (G)		24.97	0.82	0.40
Water regime (W)					
Stressed		W1	683.1	15.2	6.9
Normal		W_2	1621.7	22.5	8.6
	Lsd Water regime (W)		49.96	1.96	0.48
Phosphorus (P)					
		P_0	499.6	5.7	5.1
		P_1	1295.8	23.2	9.4
		P_2	1661.7	27.8	8.8
	Lsd Phosphorus (P)		32.17	0.92	0.48
G × W		$G_1 W_1$	349.4	15.3	6.4
		$G_2 W_1$	1016.7	15.1	7.4
		$G_1 W_2$	1438.9	26.2	9.3
		$G_2 W_2$	1804.4	18.8	7.8
	Lsd G x W		38.82	1.50	0.57
G × P		$G_1 P_0$	649.2	6.5	5.5
		G_1P_1	1046.7	28.3	10.0
		$G_1 P_2$	986.7	27.5	8.2
		G_2P_0	350.0	4.8	4.7
		G_2P_1	1545.0	18.0	8.8
		$G_2 P_2$	2336.7	28.0	9.3
	Lsd G × P		41.18	1.5	0.54
W × P		$W_1 P_0$	255.8	5.00	4.8
		$W_1 P_1$	538.3	18.2	9.0
		$W_1 P_2$	1255.0	22.5	7.0
		$W_2 P_0$	743.3	6.33	5.3
		$W_2 P_1$	2053.3	28.2	9.8
		$W_2 P_2$	2068.3	33.0	10.5
	Lsd W × P		45.01	1.55	0.42
Factor significance		**Genotype (G)**	≤ 0.001	≤ 0.001	0.15
		Water regime (W)	0.003	0.004	0.005
		Phosphorus (P)	≤ 0.001	≤ 0.001	≤ 0.001
		G × W	≤ 0.001	≤ 0.001	≤ 0.001
		W × P	≤ 0.001	≤ 0.001	≤ 0.001
		G × P	≤ 0.001	≤ 0.001	≤ 0.001

interactions ($p \leq 0.001$). Yield in Gadra was more susceptible to soil water deficit. The reductions in yield under water stress were 76% for Gadra and 44% for KE-3. Compared to treatments where no P was added, the addition of P either at 40 kg ha^{-1} or 70 kg ha^{-1} caused highly significant yield increases.

Significant genotype and P interactions in yield were observed. The increases under the 40 kg ha^{-1} and 70 kg ha^{-1} P treatments were 61 and 52% respectively for Gadra and 340 and 560% respectively for KE-3. For Gadra, the lower P application rate increased yield more than the high P application, whereas at the 70 kg ha^{-1} P level the yield declined. This reduction in yield was higher under water stress regime for the 70 kg ha^{-1} P treatment. Gadra increased yield with P addition under both water regimes only at the 40 kg ha^{-1} P treatment. It was unable to utilize the increased availability of P at the high P treatment. Significant water stress and P interactions were observed with P application reducing the negative effects associated water stress. The effect was higher in the 40 kg ha^{-1} P and less in the 70 kg ha^{-1} P treatment. Across genotypes, under water stress condition, the increases in yield were 60 and 50% respectively for the 40 and 70 kg ha^{-1} P additions. Under normal irrigation, the respective increases were close to 340 and 570%.

Also, highly significant genotype, water stress and P interactions were noted ($p \leq 0.001$) in Table 1. Figure 2A shows yield interactions between the 3 factors. Without normal water supply, yield declined in both genotypes; however, yield increased under water stress when it was coupled with P additions. Increasing soil P to 40 kg ha^{-1} increased yield in both genotypes but the yields were lower in the water stressed treatments. Further increase in P reduced yield in Gadra under water stress. The highest yields were obtained in KE-3 under normal irrigation. Application of high P (70 kg ha^{-1}) to the soil in KE-3 was able to sustain high yields even under water stress.

Effects on yield components

Yield responses to changes in phosphorus and water stress were transduced mainly through changes in yield components such as pods per plant, pod length, and 100-seed weight (Table 1). There were significant interactions between water and phosphorus, genotype and water regime, genotype and phosphorus and water, as well as genotype and phosphorus for these yield components.

Number of pods per plant

Effects of treatments on pod number are shown in Table 3. The number of pods per plant in Gadra was significantly higher than for KE-3. Water stress as previously noted with yield and vegetative components reduced the number of pods per plant, with the average reduction being 32%. Addition of P increased the number of pods highly significantly ($p \leq 0.001$) with about 300% for the 40 kg ha^{-1} P application and close to 390% in the high P treatment. The number of pods per plant showed a significant interaction between genotype and water stress treatments. Overall, water stress reduced number of pods per plant to an average of 42% in Gadra and 20% in KE-3.

There was a significant interaction between genotype and P level in pod number per plant (Table 3). P increased the number of pods per plant for Gadra by 335 and 320% for 40 and 70 kg ha^{-1} P level, respectively. Regarding KE-3, the increases were 270 and 480% for the 2 respective P treatments. Highly significant interactions between water stress and P levels were seen ($p \leq 0.001$). Increases in pod number as a result of increased soil P were observed in both water stress and normal irrigated treatments. The increases under water stress treatments were 263 and 350% for the 40 and 70 kg ha^{-1} P, respectively compared to 345 and 421% respectively under normal irrigation.

Figure 2B shows the interactive effects of genotype, water regime and P levels on pod number. There were significant genotype, water stress and soil P interaction in terms of pods per plant. Water stress reduced pod number, with the lowest pod numbers occurring in the no P addition treatment. Addition of P increased the number of pods but there were differences in response among the genotypes. For Gadra, the largest increase occurred with the 40 kg ha^{-1} P but the high P treatment (70 kg ha^{-1} P) coupled with water stress reduced pod number per plant. For KE-3, the 70 kg ha^{-1} P treatment still caused further increases of almost the same magnitude with the 40 kg ha^{-1} P treatment.

Pod length

The effects of treatments on pod length are presented in Table 3. There were no differences in pod length among the genotypes. Whereas water stress reduced pod length by an average of 19%, there were no differences among the genotypes. Phosphorus increased the length of pods significantly, with the 40 and 70 kg ha^{-1} treatments causing 85 and 72% increases respectively.

There was a significant genotype by water stress interaction for pod length. Gadra showed a 13% pod reduction from water stress whereas KE-3 had a slight increase (20%) in pod length. Significant genotype and P interactions were discerned with the 40 kg ha^{-1} P addition causing an 85% increase in pod length for Gadra, and the high P increasing the pod length by a lesser magnitude of 72%. KE-3 showed consistent increases of 90 and 100%, respectively. Water stress interacted with P significantly in pod length. Under water stress, the low P (40 kg ha^{-1}) increased pod length by a greater magnitude (86%) compared to 45% for the 70 kg ha^{-1} treatment. Under normal irrigation, the pod length increased consistently- 84% (40 kg ha^{-1} P) and 97% (70 kg ha^{-1} P), thus showing a comparatively larger response

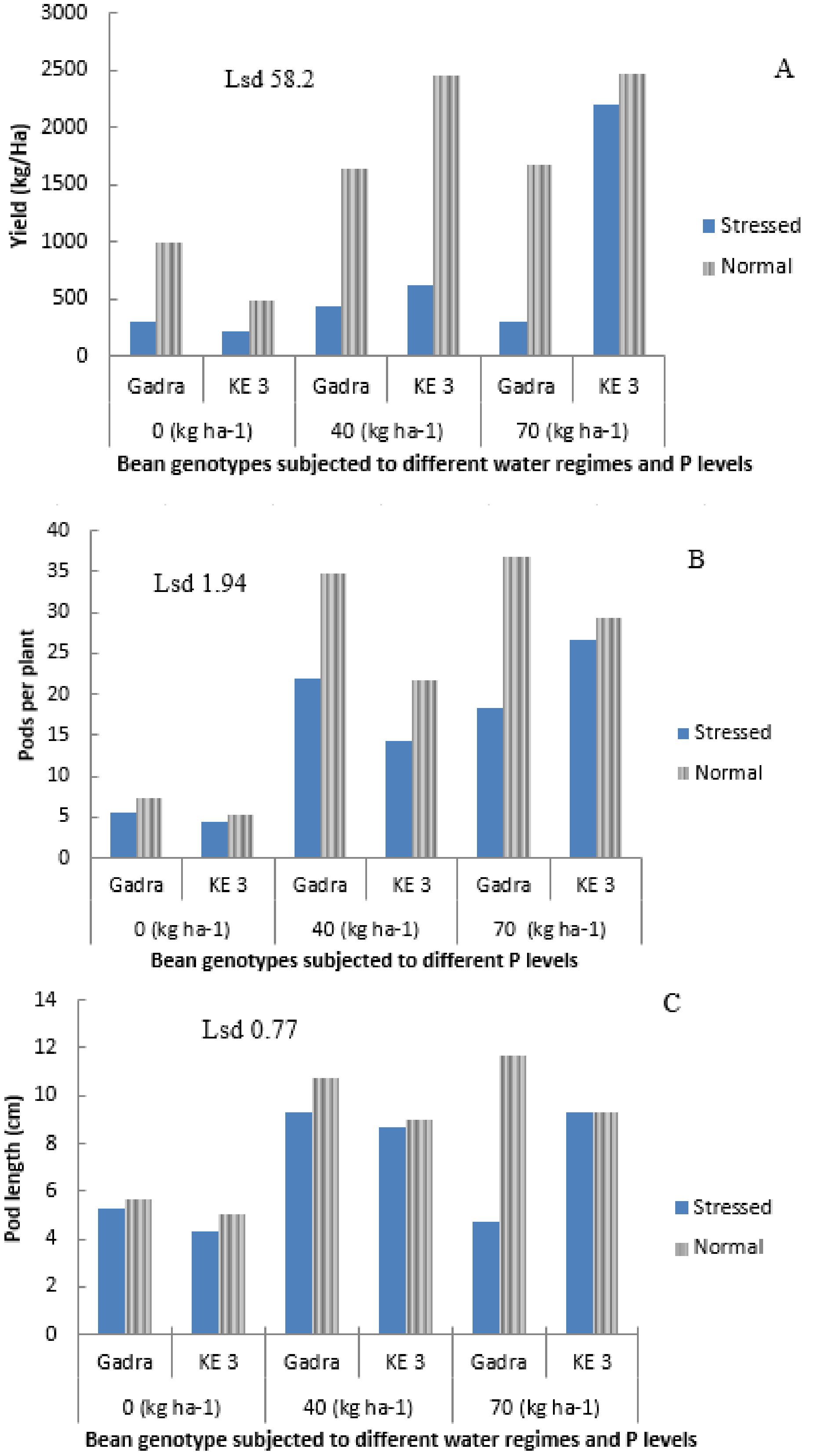

Figure 2. Three way interactive effects of genotype, water regime and phosphorus levels on yield pods per plant and pod length in common beans (*P. vulgaris* L.).

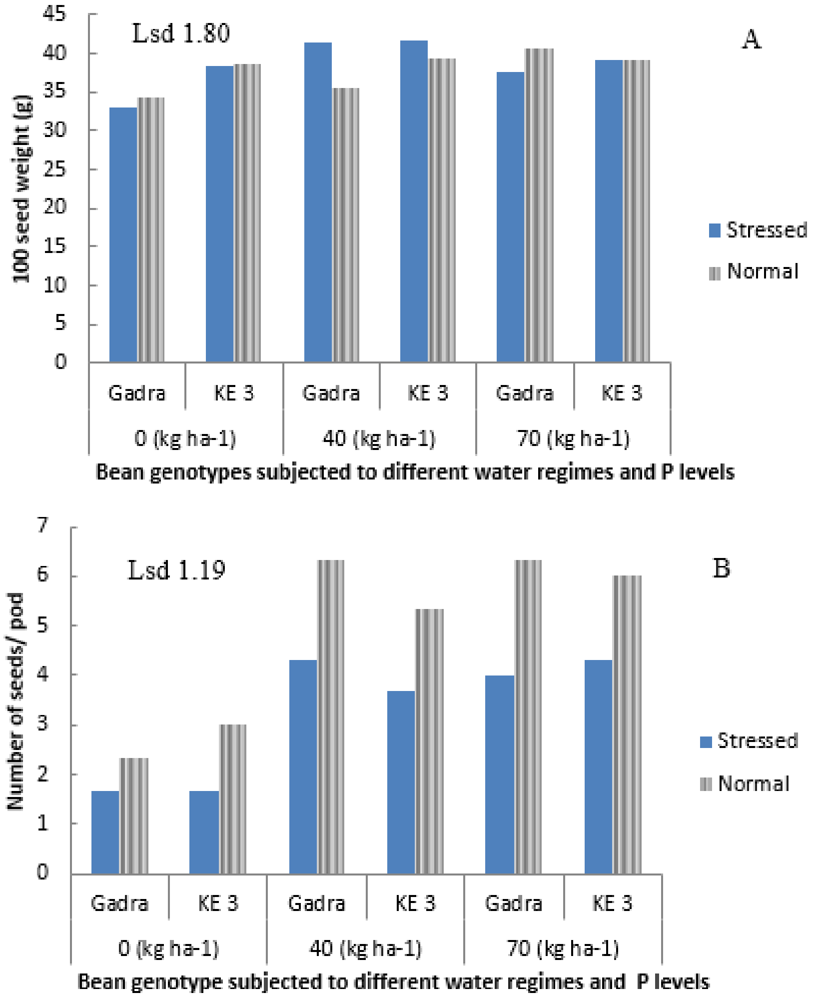

Figure 3. Three way interactive effects of genotype, water regime and phosphorus levels on seed weight, seeds per pod of common beans (*P. vulgaris* L.).

in the higher P level.

Figure 2C shows the highly significant genotype, water stress and P interaction in pod length ($p \leq 0.001$). Water stress reduced pod length; however for KE-3 at the high P level, pod length increased under the water stress treatment. Generally, P increased pod length but this was dependent on genotype and soil water status. For Gadra, increase in P occurred only in the normally irrigated plots; at the high P the pod length reduced under the water stress. KE-3 consistently increased with P, and at the high P was able to overcome the negative effect water stress so that the pods under water stress were longer compared to the no stress treatment.

Seed weight

The effect of treatments on 100-seed weight is presented in Table 1 and Figure 3A. Genotype had a highly significant effect on seed weight, with KE-3 having higher seed weight compared to Gadra. Overall, soil water stress did not have an effect on seed weight ($p = 0.24$) whereas P increased seed weight. Similar to the yield response, the lower P addition increased yield more than the high P rate. No interaction between water stress and P were observed ($p = 0.75$). There was a highly significant genotype and P interaction ($p \leq 0.001$), with Gadra having a higher response to P addition and both P addition causing about 15% increase in seed weight. KE-3 increased seed weight only under the 40 kg/ha treatment (5%). Water stress and P interacted significantly for seed weight with larger increases in seed weight under the water stressed than normal irrigated treatments. Under water stress, the 40 and 70 kg ha^{-1} P increased seed weight by 16.2 and 7.2% compared to 2.7 and 9.0%, respectively under normal irrigation. Significant genotype, water stress and P interaction were observed ($p = 0.009$) in Figure 3A. P increased seed weight higher in Gadra than in KE-3, and at high P level the negative effects of water stress were counteracted by P. For KE-3 at high P, there was no difference between water stressed and normal irrigated plots.

Number of seeds per pod

The number of seeds per pod was least sensitive to treatment effects (Table 2 and Figure 3C). Genotype did not exert a significant treatment effect on seed number per pod. Water stress significantly reduced the number of seeds per pod, whereas P caused highly significant increase in seeds per pod. The increase for both the 40 and 70 kg ha^{-1} P was the same- about 130%. These increases were reduced by water stress in both genotypes. No significant interaction was observed for either water stress by genotype, P by genotype or genotype by water stress by P level.

DISCUSSION

This study demonstrates the interrelationship between water stress tolerance and phosphorus in common beans. In particular, it highlighted impact of the simultaneous occurrence of low soil P and soil water deficit on plant development and yield. This is a common situation in many bean growing regions where drought occurs in areas that also have low soil phosphorus (Namugwanya et al., 2014). Plant responses to stress are highly complex and involve changes at the transcriptome, cellular and physiological levels (Suriyagoda et al., 2014); and evidence shows that plant response to multiple stresses differs from that of the individual stresses (Atkinson and Urwin, 2012). Muller et al. (2014) identified genes induced in response to drought stress and demonstrated the differential gene expression during flowering and grain filling in common bean grown under drought stress conditions.

Characteristically, soils become deficient in P after prolonged degradation by erosion and repeated removal in crop harvest without replacement by fertilization of removed P (Henao and Baanante, 2006). The advent of climate change has increased the frequency and extent of extreme weather patterns including drought (Godfray et al., 2010; Jin et al., 2014). Our study demonstrated that in cases where P deficiency and water stress occur simultaneously, addition of P to the soil can increase the robustness of plants thereby increasing biomass and ultimately seed yield. This ameliorates the negative effects of soil water deficits. Previous studies have shown that these responses are mediated through either increased phosphorus acquisition efficiency (PAE) and/or phosphorus use efficiency (PUE) (Atemkeng et al., 2011; Cichy et al., 2009; Vandamme et al., 2016). The former invariably involves modification in root architecture and the latter able to produce biomass even under limited soil P. The two genotypes used in the current study exhibited differences in responses to low P and soil moisture stress. KE-3 appeared to be more efficient at using P under water stress especially at the high P level but Gadra was more efficient only under normal irrigation supply and low (40 kg ha^{-1}) P addition. Another difference was in responses on the number of pods per plant and pod length. P increased the number of pods per plant, as for Gadra, the increase was more at the low P addition (40 kg ha^{-1} P) and under non-water limiting conditions. For KE-3, the increases in pod numbers were moderate at low P (270%) and particularly high at the high P addition (480%), and were still reasonably high even under low soil moisture conditions. The results were similar for pod length. Mndolwa et al. (2018) who evaluated P responses to common beans and used almost similar P application rates (50 kg and 100 kg ha^{-1} P) reported similar findings. These authors observed increased biomass and seed yield associated with increases in soil P and suggested improved PUE as the main reason for the improvement.

In their work on evaluation of P- efficient germplasm in tropical regions, Beebe et al. (2006) suggested that genotypes selected for adaptation to low- P soils may be more sensitive to drought. This may be due to their predominantly shallow branching characteristic that allows for more efficient soil foraging but prevents them from accessing water in lower soil horizons (Nielsen et al., 2001). We noted that Gadra that was reported to be drought susceptible (Kalima, 2013) had a better PUE. Studies on cowpea (*Vigna unguiculata*), a close relative of common beans have shown that yield in legumes is strongly dependent on water supply during reproductive phase with less influence of vegetative phase water deprivation (Ziska and Hall, 1983; Hall, 1999). Better performance of plants under soil water deficits when P is adequate is thought to be due to increased water use efficiency (Suriyagoda et al., 2010). Additionally, it has been reported that among other traits seed yield is positively correlated with ability to maintain high leaf chlorophyll content (Ambachew et al., 2015). Nielsen et al. (2001) showed that under low P, inefficient genotypes utilise more of their net carbon assimilation on root respiration thus further reducing carbon availability for maintenance, construction of plant tissues and ion uptake.

Lynch et al. (1991) postulated that P availability affects bean growth primarily through effects on leaf appearance and biomass partitioning between photosynthetic and respiring organs, rather than through effects on leaf photosynthesis. The days to full maturity can be used as a proxy for green leaf duration because after this period the leaves begin to lose their chlorophyll. The green leaf duration is an important phase in annual crops as it is the period in which the crop actively synthesizes photo assimilates and uses them for general development or accumulates them in reserve for later remobilization in peak demand (Sadras and Tripani, 1999). Our results showed that water stress generally reduced the days to full maturity (DFM), and the decrease was higher in Gadra (21%) compared to KE (10%). Highly significant genotype and phosphorus interactions were observed. Although P addition increased the DFM, KE-3 had a significantly larger increase compared to Gadra. DFM increased with increase in P addition but these increases occurred only where irrigation was normal. For Gadra, water stress reduced DFM when accompanied with P addition at the 70 kg ha^{-1} P level. On the other hand, KE-3 showed consistent increase in DFM with more P addition. The effect of low soil P shortening DFM related to early onset of senescence. Common beans are annual plant with typical monocarpic type of senescence, where the plant dies entirely after formation of seeds and fruits. The key processes involved in senescence are changes in chlorophyll, proteins and consequently changes in photosynthesis and respiration rates (Mataa et al*.,* 2018). Drought escape mechanisms involve rapid phenological development, early flowering and maturity (Namugwanya et al., 2014). Both genotypes exhibited longer DFM under high soil P. It is possible that low P in the plant initiated early senescence or it may just be a stress response where reproductive phase is initiated early to ensure seed production before the plant dies. In either case, the grain filling period was reduced and the overall yield lowered compared to optimal soil P treatments.

This study did not analyse root development, therefore, we can only postulate that Gadra which had more yield robustness under low soil phosphorus may have a shallow and adventitious root system and was therefore able to forage for phosphorus more efficiently under low P soils. Although root architectural traits that increase topsoil foraging are advantageous for phosphorus acquisition they may incur trade-offs for the acquisition of deep soil resources such as water (Ho et al*.,* 2005). These same authors suggested that under low soil phosphorus, shallow rooted genotypes grow best, whereas under drought stress, deep rooted genotypes grew best. It has been further reported that adventitious roots have lower metabolic cost than basal roots and P efficient genotypes have lower root respiration rates (Nielsen et al*.,* 2001). It has also been reported that in some species, P efficient genotypes under low soil P exhibit increased root aeronchyma which may decrease root respiration (Galindo-Casteñeda et al., 2018). Boutraa (2009) and Liao et al*.* (2004) suggested that improved PUE is related to the capacity of the plant to accumulate dry matter despite inadequacy of soil P. Such genotypes are able to produce more pods and seeds than non-low P tolerant genotypes (Atemkeng et al*.,* 2011; Boutraa, 2009). In our study, high production of pods was clearly demonstrated in the genotype Gadra at the high soil P rate (at 40 kg ha^{-1}) where the number of pods increased under the normal irrigation treatments.

Phosphorus improved crop performance under low soil moisture conditions or drought conditions. According to Frahm et al*.* (2004) and Beebe et al. (2013), drought tolerance mechanisms include capacity to avoid dehydration, while preserving comparatively high tissue water potential, which is partly due to improved root length, density and depth that maximises the available soil moisture for uptake by the crop. Another mechanism is through escape mechanism which is the ability of the plant to complete its life cycle before onset of harsh soil moisture deficits (Beebe et al*.,* 2013). Liu et al*.* (2017), working on dwarf bamboo showed that P application enhanced leaf photochemical activity, increased chlorophyll content, reduced thermal dissipation, increased scavenging of reactive oxygen species and reduced lipid peroxidation in water-stressed plants.

It is interesting to note that this apparently more P efficient genotype developed P toxicity symptoms at high soil P as evidenced by reduction in biomass. Shane et al. (2004) reported that P toxicity symptoms include growth reduction, early leaf senescence and leaf chlorosis. It has been suggested that P toxicity develops when P uptake exceeds utilization (Shane et al., 2004) and is more evident in slow growing or smaller genotypes (Shane et al., 2003). Gadra had less shoot biomass and shorter plant height. KE-3 responded more positively to both P application treatments, increasing plant height by 35% (low P) and 82% (high P).

Conclusion

The ability of P to increase yield was demonstrated even under soil water deficits. Phosphorus significantly increased grain yields mainly through increasing the number of pods per plant and seed weight. However, there was a tradeoff between ability to forage for P under low soil conditions and ability to tolerate and efficiently utilise high P applications. Thus, the genotype that exhibited higher yield at low soil P possibly due to higher P foraging was not able to utilise high applications of P to increase grain yields and actually showed P phytotoxicity. Our results showed the benefits of P application but suggest that attempts to improve yield by high application of P fertiliser particularly under soil water deficits should consider genotype morphology, the ability and efficiency to utilise the phosphorus.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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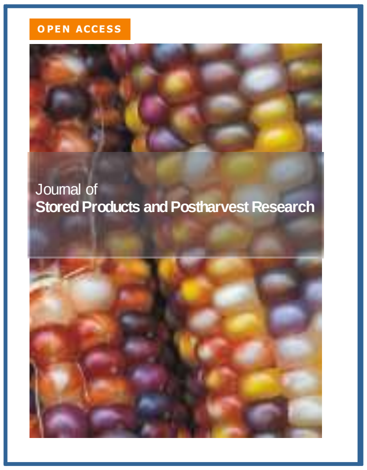

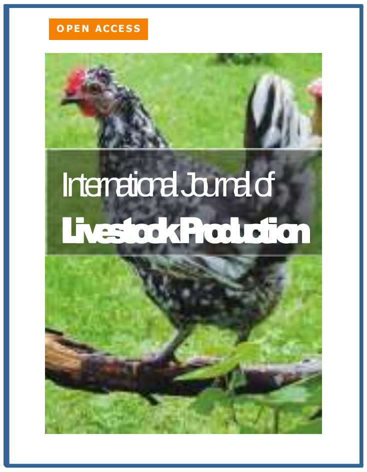

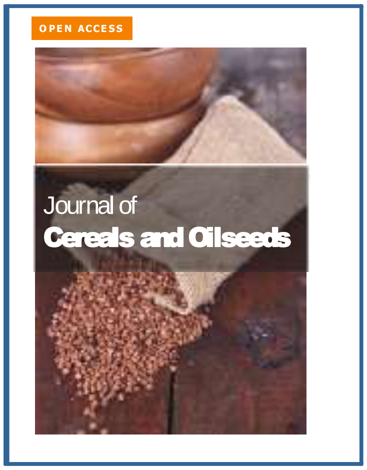

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