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Full Length Research Paper

Study on major causes of chicken mortality and associated risk factors in Bahir Dar Zuria District, Ethiopia

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A cross sectional study was conducted to assess major causes of chicken mortality and associated risk factors from November 2013 to May 2014 in Bahir Dar Zuria District, Ethiopia. One hundred respondents were selected using simple random sampling technique. Data collected using questionnaire survey and from laboratory investigation of parasites were analyzed using STATA version 11. Among all respondents, 63 and 37% of the respondent used extensive/backyard and small-scale intensive poultry production systems, respectively. All respondents provided housing for their chicken under small-scale intensive system, while 96.8% provided housing under extensive production system. All small-scale intensive producers and 88.8% of extensive producers practiced house cleaning practices. About 56 and 5% of the respondents provided water as free accesses in small scale intensive and extensive production systems, respectively. Provision of commercial feed was practiced only by small scale intensive poultry producers. Presence of diseases, feed shortage, predators and bad weather condition/extreme weather condition/ were identified as the major causes of chicken mortality. Among diseases Newcastle diseases, Infectious bursal diseases and coccidiosis were cited in their order of importance. Among 69 fecal samples collected 44 (69.84%) were positive for nematodes, cestode and protozoal parasites. High mortality rates were recorded in both production systems. A 50% under extensive and 36% under small-scale intensive production systems, poultry producers dispose dead birds due to different diseases by throwing elsewhere near the farm/backyard area. Among all respondents, 24% vaccinated their chicken, whereas 76% did not practice vaccination to common diseases. Thus, poultry improvement program in the area should focus on minimizing and ultimately avoiding constraints of poultry sector to see the required performance at the expected level.

Key words: Chicken mortality, production systems and management practices.

INTRODUCTION

Rural poultry production is an important agricultural activity Africa providing scarce animal protein resource in the form of meat and eggs as well as being a reliable of pet each. Village chickens also fulfill a number of functions for which it is difficult to assign any monetary value (Alders and Spradbrow, 2000). According to

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Ministry of Agriculture, Ethiopia has a large population of chickens estimated about to be 48.89 million (CSA, 2011), reared in all agro-ecological zones of the country predominantly under traditional husbandry system. Poultry husbandry in an intensive system is also practical in some urban and per urban areas and only represents 1% of the total population in the country (Demelash and Ajebu, 2003).

The poultry sector in Ethiopia can be characterized into village or backyard, small scale and commercial poultry production system (Dawit et al., 2008). Backyard poultry production is the predominant system in Ethiopia which accounts for nearly 99% of the poultry population consisting mainly of local chicken breeds under individual farm household management. It is also common to find a few exotic breeds distributed through the extension programs in the backyard production system. The small-scale intensive poultry production system comprises a flock size ranging from 50 to 500 exotic breeds' operation commercial bases and outdoor with a low bio-security level. Commercial poultry production system is highly intensive production system that involves greater than 10,000 birds kept under indoor and heavily depends on imported breeds (Dawit et al., 2008).

In Ethiopia, the poultry sector has been adversely affected by a variety of constraints; of these poultry diseases continues to play the major role hampering its development. Poultry mortalities due to diseases are estimated to range from 20 to 50%, but it may rise as high as 80% during epidemics (Tadelle and Ogle, 2001). Diseases, feed shortage, bad /extreme whether condition and predation were the major constraints in all areas surveyed under village production system. The impact of diseases includes lost revenue, vaccination cost (prevention, eradication, decontamination and restocking) (Safari et al., 2004). Chick's mortality represents a major loss in scavenging village chicken production system in Ethiopia. Several reasons for the high mortality and low productivity have been suggested such as mismanagement, malnutrition, disease and predation (Negesse, 1991). Newcastle disease (Serkalem et al., 2005) and predator attack (Halima, 2007) have also been reported as a major constraints to chicken production in central and Northwest, Ethiopia. Thus, there are many constraints to the development of small holder's poultry production that need to be addressed. These comprise disease control, protection against various predators, better feeding, genetic improvement, marketing, training and management access to production imputes, infra-structural and capital farmer organization, and for most conducive institution and governmental policies (Aklilu et al., 2004).

There is an increased demand for poultry and poultry products in Bahir Dar Zuria District, due to increased population growth and urbanization. The number of people involved in the small-scale intensive and extensive chicken production increased dramatically in the vicinity of Bahir Dar. This growth effort also supported by government extension programs through distribution of day old chicks and pullets to small scale intensive and village chicken producers. Despite the effort by small-holder farmers and government to enhance the sector, there are several constraints affecting the poultry sector not to perform at the expected level. Thus, there is a need to develop a systematic study conducted to identify the major causes of chicken mortality and propose feasible interventions in Bahir Dar Zuria District, Ethiopia. Thus, the objectives of this research work was to identify causes of chicken mortality and associated risk factors and recommend possible intervention options to reduce chicken mortality and enhance economic contribution in Bahir Dar Zuria District.

MATERIALS AND METHODS

Description of study area

The study was carried out in Bahir Dar Zuria District, which surrounds the capital city of Amhara regional state, Bahir Dar. It is located at 565 km northwest of Adiss Ababa. The center is located 11° 29' N latitude and 37° 29' E longitudes with an elevation of 1730 m.a.s. l. It receives a summer rainfall where the highest rainfall is between June and September and winter dry season (December to March). Its average annual rainfall is 1150 mm with temperatures ranging from 6.5 to 30°C.

Sample size determination and selection of study households

A total of 100 households were included in the study according to the formula given by Arsham (2002), $N=0.25/SE^2$, Where, N= Sample size, SE= Standard error. As a standard error of 0.05 was taken to calculate the total households to be involved in the questionnaire survey. $N=0.025/ (0.05)^2 =100$. Selection of households was in collaboration with Bahir Dar Zuria District Agricultural Office livestock experts from a total of 10 Peasant Associations (PAS) a list of 300 households were used as sampling frame. Then, using simple random sampling 100 households were selected to be included in the study.

Faecal sample collection and isolation of parasite eggs

A total of 69 feacal samples, 31 from exotic chicken under small scale intensive and 38 form local chicken under extensive/backyard production systems were collected during the study period and transported to Bhirdar Zuria District veterinary clinic using coded formalin containing bottles. Faecal samples were collected by inserting the applicator finger into the cloaca of the selected chicken to isolate eggs of gastro- intestinal nematodes and protozoas. Parasite eggs were identified using simple faecal floatation method developed by Zajac and Conboy (2006).

Procedures

Upon arrival to the laboratory, samples were kept at low temperature until processing. Floatation fluid was prepared using 400 g Nacl and 1000 ml water. The solution was stirred to dissolve adequately. Each coded faecal samples were mixed well with the floatation fluid and strained with tea strainer. Then, after removal of faecal, the debris and solution was poured in to coded test tubes.

Table 1. Characteristics respondents and production systems in the study area.

Sex of respondents	Percent (N=100)
Male	62
Female	38
Educational status of respondents	
Illiterate	36
Primary School	32
Secondary School	23
College and University	9
Age of respondents	
Young	21
Adult	79
Flock Composition Kept	
Exotic Chicken	43
Local Chicken	54
Crossbreds	3
Production Systems Used	
Extensive/Backyard	63
Small scale Intensive	37

After putting the test tubes on the rack, it was allowed to stay for 20 min according to the procedure developed by Zajac and Conboy (2006). Then, a drop of fluid from the supernatant was taken to prepare a smear and examine under microscope to identify parasite eggs.

Study populations

The populations studied were exotic chickens distributed by the government, crossbreds and local chickens (ecotypes) in Bahir Dar Zuria District.

Data collection and analysis

Household information, type of chicken reared, management, health and vaccination status of chickens, constraints, causes of mortality and common poultry diseases occurring in the area was collected using the questionnaire prepared for the survey. In addition to questionnaire data collection, general house inspection, feeding and health of poultry were carried out. Parasite infection presence and absence was also recorded. Eggs of the parasite identified helped to identify the most common parasitic diseases in the area. Data collected were analyzed using Stata software version 11 (STATA, 1999). Data of household characteristics, management practices (housing, feeding, watering), health care practices and common causes of mortality was summarized using descriptive statistics. Pearson's chi-square (χ^2) was used to determine the effect vaccination on chicken mortality.

RESULTS AND DISCUSSION

The household characteristics of the respondents (Table1) revealed that higher proportion of male Respondents were higher than females. However, in others studies conducted at small scale and extensive level females dominates in poultry production as it was reported by Upton (2004), Muchadeyi et al. (2007) and Tadesse et al. (2013).

In the present study, higher number of respondents studied at primary and secondary school and College/University level in comparison with those reported by Halima (2007) and Moges et al. (2010). In Bahir Dar Zuria District 63% of farmers used the extensive/ backyard production system, however, considering as the whole Ethiopia 99% of chickens are raised under the traditional backyard system of management (Ashenafi and Eshetu, 2004). From the total of respondents 37% of the respondents used small scale intensive production (Table 1), which represents an emerging system in urban peri-urban areas of Ethiopia (Solomon, 2008).

Housing protects birds from harsh weather condition, predators and facilitates management of chicken. In the present study, under extensive system, nearly 97% of the respondents provide night shelter while about 57% provide daytime shelter for their chicken (Table 2), this is relatively higher than the findings of Moges et al. (2010) who reported 77.9% of village chicken owners provided night shelter in Bure District North West Amhara region. All small-scale intensive producers practiced cleaning of poultry house, whereas 88.8% respondents practiced poultry house cleaning under extensive system, this agreed with the finding of Khandait et al. (2011) who reported 84. 17% house cleaning practice in Bhandara

Table 2. Housing practices under intensive and extensive production systems.

House sanitation practices	Production systems used	
	Small scale intensive (N=37)	Extensive/backyard (N=63)
House cleaning practiced	37(100%)	56 (88.8%)
Chemicals use for house cleaning	24 (64.8%)	13 (35.2%)
Housing practices specific to extensive/backyard production system (N=63)		
Provision of night shelter		61(96.8%)
Provision of day time shelter		36(57.1%)

District of India. Higher proportion of respondents used chemicals including sodium chloride, diazinon and 5% chlorine for house cleaning under small-scale intensive than extensive production system.

Generally, in the present study the use of river and borehole as water source indicate lack of clean water source (pipe water) in Bahir Dar Zuria District (Table 3). In small-scale intensive system, majority of the respondents used pipe water (70%) as water source, while the rest used river and borehole water, similar watering practices was reported by Mengesha et al. (2011) in Jamma District, South Wollo and Moges et al. (2010) in Bure District, North West Ethiopia.

The current study disclosed that under extensive system, nearly 35% of the respondents used river as water source this is not in agreement with Tadesse et al. (2013) who reported (1.1%) in Ada'a and none of village chicken owners in Lume Districts used river as a water source. The same author reported free access provision of water in 96% village chicken owners in Ada'a and Lume Districts of Oromiya, which significantly higher in comparison with the current study. Feed constitute the major cost in poultry production, to make full use of the productive potential of chicken, nutritionally balanced diet should be provided. Provision of supplementary feed was practiced by small-scale intensive and extensive systems, where as there was no provision commercial feed under extensive system (Table 3). Lack and availability could be the factor that restrained farmers' not to use commercial poultry feeds in the area. As scavenging laying hen can find approximately 60 to 70% of their feed requirement (Rahman et al., 1997), providing supplementary feeds could help to express the laying potential of chickens at village level. In small-scale intensive system, above 62% of the respondents fed their chicken commercial diet, while under extensive system 73% of the respondents provided feed in addition to the common scavenging system. However, in the current study, there was no record of using scavenging for small-scale intensive and provision of commercial diet for extensive/backyard system.

In both production systems, diseases were identified as major causes of chicken mortality, this is in agreement with the finding of Moges et al. *(*2010) in North West of Amhara Region. Predators are also cited as causes of mortality in small-scale intensive and extensive systems, while nearly 6% respondents mentioned bad weather as cause of chicken mortality (Table 4).

Cats and wild birds were identified as major predators for small scale intensive and backyard production systems, respectively. In other studies conducted by Tadesse et al. (2013) in Ada'a and Lume District, feed shortage cited as the third challenge and Melkamu and Wube (2013) cited predator as the primary causes of chicken mortality in Gonder Zuria District, Ethiopia. Birds of prey locally called "Culullee", cats and dogs and wild animals were identified as main chicken predator in rift valley of Oromyia, Ethiopia (Dinka et al., 2010). Mekonnen (2007) also reported a prey of snakes, rats, dogs and foxes in young birds in Southern, Ethiopia. This could indicate the presence of different agro-ecology could determine the presence of different chicken predators.

Faecal egg isolation and identification

Among 69 faecal samples collected 44 (63.78%) were identified positive for nematode, cestode and protozoan parasites. The major isolates were coccidian spp. (47.73%) and the other isolates were *heterakis galinarum, ralientina cesticillus*, subulura spp., capilaria spp., and prostlogonium spp (Table 5). Provision of training for farmers would help farmers to use the available feed resources at hand to enhances the economic contribution of the poultry sector. More than half (56.75%) of the respondents provided training on health care management of poultry under small scale intensive, while majority of the respondents (96.83%) respondents did not get training under extensive production systems (Table 6). This is comparable with the finding of Tadesse et al. (2013) who reported (47.2%) chicken owners provide poultry training in Ada'a and Lume District of Oromyia. Higher proportion of respondents got veterinary service in small-scale intensive (83.78%) than extensive (55.55%) production systems. The relative higher number of birds/area and convenience to provide treatment in small-scale intensive

Table 3. Chicken feeding and watering practices in Bahir Dar Zuria District.

Water sources used	Production systems	
	Small scale intensive (N=37)	Extensive/Backyard/ (N=63)
River	7 (18.92%)	22 (34.92%)
Pipe water	26 (70.27%)	28 (44.44%)
Borehole water	4 (10.81%)	13 (20.64%)
Frequency of watering		
Free Access	21(56.76%)	3(4.76%)
Two times/day	12 (32.43%)	42 (66.66%)
Once/day	4 (10.81%)	18 (28.58%)
Provision of additional feed		
Only scavenging	0 (0.00%)	17 (26.98%)
Provision of additional feed	14 (37.84%)	46 (73.02%)
Use of Commercial feed	23 (62.16%)	0 (0.00%)

Table 4. Major causes chicken mortality in Bahir Dar Zuria District.

Mortality causes	Production system used	
	Small scale intensive (N=37)	Extensive/backyard (N=63)
Diseases	24 (64.86%)	29 (46.03%)
Feed shortage	9(24.32%)	23 (36.50%)
Predators	2(5.40%)	7 (11.11%)
Bad weather Condition	2(5.40%)	4 (6.34%)
Total	37	63
Common mortality cause of diseases origin in their priority order		
Newcastle Diseases	22(59.45%)	35(55.55%)
Infectious Bursal Disease	9(24.32%)	22(34.92%)
Coccidiosis	6(16.22%)	6(9.52%)
Total	37	63

Table 5. Parasite eggs identified during the study from study chicken populations.

Parasitic eggs identified	Number of positive (N=44)
Coccidia Spp.	21(47.73%)
Heterakis galinarum	9 (20.45%)
Ascardia galli	6(13.64%)
Railientina cesticillus	3 (6.82%)
Subulura spp.	2 (4.54%)
Cappilaria Spp.	2(4.54%)
Prostlogonium Spp.	1(2.27%)
Total	44(100%)

than the backyard producers might lead farmers to seek more frequent veterinarian attention in small scale intensive production system. Provision of adequate veterinary services is also mentioned as major problem under extensive system as reported by Tadesse et al. (2013) and Mengesha et al. (2011).

Table 6. Health care practices followed in Bahir Dar Zuria District.

Health practices	Production system used	
	Small scale intensive (N=37)	Extensive/Backyard (N=63)
Provision of training	21(56.75%)	2(3.17%)
Provision of Veterinary Services	31 (83.78)	35(55.55%)

Table 7. Chicken mortality and disposal methods of dead chicken in and around Bahir Dar Zuria District.

Production system used			Disposal methods of dead chicken			Total
			Throwing elsewhere	Burying	Burning	
Extensive/Backyard	Mortality (N=63)	High (96.82%)	50(82%)	11(18%)	0.0%	61(100%)
		Low (3.17%)	1(50%)	1(50%)	0.0%	2(100%)
Small scale intensive	Mortality (N=37)	High (97.29%)	13(36%0)	20(55.6%)	3(8.3%)	36(100%)
		Low (2.70%)	0.0%	1(100%)	0.0%	1(100%)

More than half of the respondents of small scale poultry producers got adequate training on poultry rearing. However, a very few number of respondents got training on poultry training under extensive system and this might suggest to increase the number of trainings to be provided for village chicken owners. In other studies, relative higher number of farmers provided training and extension services in Ada'a and Lume District of East Shewa and Jamma District of South Wollo as reported by Tadesse et al. (2013 and Mengesha et al. (2011), respectively.

Nearly 97% of respondents in both small-scale intensive and extensive production systems experienced high mortality, while very few nearly 3% experienced lower mortality (Table 7). High chicken mortality was also mentioned as common problem in other studies conducted on village chicken by Tadelle (2001); Tadelle et al. (2003); Nigussie et al. (2003) and Serkalem et al. (2005). Disposing dead chickens using methods such as burying and burning could help farmers to prevent the transmission of the diseases into new flock and enhances the overall bio-security in small-scale producers. According to the present study 82% of the respondents used throwing elsewhere, while 18% used burying to disposed dead chicken under the backyard system (Table 8). None of the respondents used burning under extensive system. Burying is unlikely to pose environmental impact (Ritter and Chirnside, 1995).

Under small-scale intensive system 55.6 and 8.3% of the respondents used burying and burning methods to dispose dead chicken. Even though more than half the respondents used proper disposal methods of dead chicken, 36% of the respondents throw dead birds elsewhere near the farm. Burning/incineration process is expected to destroy all infective agents (NABC, 2004). The practice of throwing of dead chicken elsewhere in both production systems might indicate the need for further awareness of the farmers about the role using proper disposal method in prevention of infectious poultry diseases. Farmers should be aware about merits and drawbacks different disposal methods currently available (Ritter and Chirnside, 1995). Provision of further training on farm bio-security including disposal dead chicken would be beneficial as it could reduce the transmission of disease agents. Among respondents 24% vaccinated and 76% did not vaccinate for common diseases in the area. In other studies on village chickens conducted by Moges et al. (2010a); Leta and Endalew (2010); Mengesha et al. (2011) and Takele and Oli (2011) there was no record of village chicken vaccination practice by farmers in different parts of the country at village level.

In the current study, however, the mortality of chicken in vaccinated and non-vaccinated flocks did not show a significant difference ($p>0.05$). Most vaccines are sensitive to heat; an adequate cold-chain system often has to be created and maintained to preserve the quality of a vaccine before it is administered (Galazka et al., 1998). Thus, the high mortality in the vaccinated chicken could indicate that the vaccines used might not be maintained in suitable cold chain before providing for the chickens.

Conclusion

Despite the socio-economic role of chicken in the study area, diseases were cited as the major cause for chicken loss, followed by feed shortage, attack of predators among other factors. Thus, the poultry improvement program in the area should focus on minimizing and

Table 8. Chi Square analysis of provision of vaccination and mortality.

Vaccination status		Mortality		X^2	P-value
		High	Low		
Vaccinated	24	23(95.8%)	1(4.2%)	0.148	0.701
Not vaccinated	76	74(97.4%)	2(2.6%)		

ultimately avoiding these challenges. Thus, if the economic contribution of chicken is required to perform at the expected level, provision of a hand on training on importance of providing additional feed (about 27% practiced at backyard), provision of free access watering (practiced by about 57% small-scale intensive and 5% under extensive), housing and health care of chicken would have a paramount importance.

RECOMMENDATIONS

(i) Training and extension service provision should address farmers involved at extensive, backyard level;
(ii) Use of river and borehole water could expose chicken for infection, thus government should establish clean water supply so that farmers can provide clean water for their chicken;
(iii) The use of locally available underutilized feed resources should be considered to reduce feed shortage;
(iv) Awareness should be created on proper disposal methods of dead chicken for the farmers.

Conflict of Interest

The authors have not declared any conflict of interest.

REFERENCES

Aklilu HA, Alemkinders CJM, Udo HMJ (2004). Socio-economic factors affecting poultry keeping in Ethiopia and their implication for innovation research approaches, department of ARWS, MU, Mekelle, Ethiopia.

Alders R, Spradbrow P (2000). Newcastle Disease in village chickens, A field manual. Maputo, Mozambique P. 46.

Arsham H (2002). Descriptive sampling data analysis. statistical thinking for managerial decision making. Retrieved November 30, 2011, from http://ubmail.ubalt.edu/harsham/Business-stat/opre504.htm#rwhyrssm .

Ashenafi H, Eshetu Y (2004). Study on Gastrointestinal Helminths of Local Chickens in Central Ethiopia. Revue Méd. Vét. 155:504-507.

Dawit A, Tamrat D, Stotaw F, Nzietcheung S, Roy D (2008). Overview and background paper on Ethiopia's poultry sector. Relevance for HPAI Reseasrch in Ethiopia. www.hpai-reserach net. Accessed 06 April 2011.

Demelsh B, Ajebu N (2003). Observation into the effect of outbreak of coccidiosis associated with respiratory infection at poultry farm of Awassa College of Agriculture. Ethiop. Vet. J. 7:32-45.

Dinka H, Regassa C, Fufa D, Endale B, Leta S (2010). Major Constraints and Health Management of Village Poultry Production in Rift Valley of Oromia, Ethiopia. American-Eurasian J. Agric. Environ. Sci. 9(5):529-533.

Galazka A, Milstien J, Zaffran M (1998). Thermostability of vaccines. Geneva: World Health Organization; 1998.

Halima H (2007). Phenotypic and Genetic Characterization of Indigenous Chicken Populations in Northwest Ethiopia. PhD Thesis; University of the Free State, Bloemfontein, South Africa, P. 186.

Khandait V, Gawande S, Lohakare A, Dhenge S (2011). Adoption Level and Constraints in Backyard Poultry Rearing Practices at Bhandara District of Maharashtra (India). Res. J. Agric. Sci. 2(1):110-113.

Leta S, Endalew B (2010). Survey on Village Based Chicken Production and Utilization System in Mid Rift Valley of Oromia, Ethiopia. Global Veterinaria, 5(4):198-203.

Mekonnen G (2007). Characterization of the smallholder poultry production and marketing system of dale, wonsho and loka abaya weredas of SNNPRS. MSc Thesis. Hawassa University.

Melkamu Y, Wube A (2013). Constarints and opportunities of village chicken production in Debsan Tikara Kebele, Gondder Zuria Woreda. Int. J. Sci. Res. Pub. 3 (9):2250-3153.

Mengesha M, Tamir B, Dessie T (2011). Village Chicken Constraints and Traditional Management Practices in Jamma District, South Wollo, Ethiopia. Lives. Res. For. Rural Dev. 23(37). Retrieved from:http://www.lrrd.org/lrrd23/2/meng23037.htm.

Moges F, Abera M, Tadelle D (2010). Assessment of village chicken production system and evaluation of the productive and reproductive performance of local chicken ecotype in Bure district, North West Ethiopia. Afr. J. Agri. Res. 5(13):1739-1748.

Muchadeyi F, Wollny C, Eding H, Weigend S, Makuza M, Simianer H (2007). Variation in village chicken production systems among agro-ecological zones of Zimbabwe. Trop. Anim. Health. Prod. 39:453-461. http://dx.doi.org/10.1007/s11250-007-9050-0 PMid:17966277

NABC (2004). Carcass disposal: a comprehensive review. Report written for the USDA Animal and Plant Health Inspection Service. National Agricultural Biosecurity Centre, Kansas State University, USA.

Negesse T (1991). A survey of internal parasites of local chickens of southern Ethiopa. Indian J. Poult. Sci. 26:128-129.

Nigussie D, Alemu Y, Tadelle D, Samuel W (2003). On-station and on-farm evaluation of the 'hay-Box chick brooder' using different insulation materials at Debre Zeit Agricultural Research Center and Denbi village, Adaa woreda. In: Proceedings of the 10th annual conference of the Ethiopian Society of Animal Production (ESAP), August 21–23, held in Addis Ababa, Ethiopia. pp. 211–216.

Rahman M, Sorensen P, Jensen H, Dolberg F (1997). Exotic hens under Semi-scavenging condition in Bangladesh. Livestock Research for rural Development, 9:3. Retrieved from:http://www.cipav.org.co/ilrrd/ilrrd931.htm.

Ritter WF, Chirnside AEM (1995). Impact of dead bird disposal pits on groundwater quality on the Delmarva Peninsula. Bioresource Technol. 53:105–111. http://dx.doi.org/10.1016/0960-8524(95)00057-L

Serkalem T, Hagos A, Zeleke A (2005). Seroprevalence study of Newcastle disease on local chickens in central Ethiopia,FVM, AAU, DebreZeit, Ethiopia.

Solomon D (2008): Ethiopia: Poultry sector country review. FAO, Rome, Italy. ftp://ftp.fao.org/docrep/fao/011/ai320e/ai320e00.pdf.

Tadelle D (2001). The role of scavenging poultry in integrated farming systems in Ethiopia. Debre Zeit Agricultural Research Center, Debre Zeit, Ethiopia. Livestock feed resources within 55 integrated farming

systems. pp. 377–399. (Available from http://www.fao.org/Ag/againfo/resources/documents/frg/conf96pdf
Tadelle D, Ogle B (2001). Village poultry production systems in central highlands of Ethiopia. Trop. Anim. Health Prod. 33(6):521-537.
Tadesse D, Harpal S, Ashenafi M, Wondimeneh E, Tadelle D (2013). Study on management practices and marketing systems of village chicken in East Shewa, Ethiopia. Afr. J. Agric. Res. 8(22):2696-2702, 13 June, 2013.
Takele T, Oli W (2011). Uses and flock management practices of scavenging chickens in Wolaita Zone of southern Ethiopia. Trop. Anim. Health Prod. 44:537-544.
Upton M (2004). The Role of Livestock in Economic Development and Poverty Reduction Online.Rome(Italy).FAO.http://www.fao.org/ag/againfo/projects/en/pplpi/docarc/wp10.pdf> Accessed 2012 March 10.
Zajac A, Conboy G (2006). Veterinary clinical parasitology . 7th edition, Balackwell publishing, Ames, Lowa. P. 4-6.

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Physiological deterioration of pigeon pea seeds during storage

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The pigeon pea appears as a good option for farmers to be used directly in food and feed and/or green manure. In spite of this potential, its cultivation area in our conditions is still incipient, mostly due to the lack of seeds of superior quality. It is known that the process of physiological deterioration of seeds varies according to species, constituting a relevant factor in seed technology. Storage problems in Brazil are still an issue and the decrease of physiological quality of seeds at this stage is considerable, especially for seeds containing high concentrations of protein (> 22%) as for the pigeon pea. In this context, this study aimed to evaluate the physiological deterioration of pigeon pea seeds stored in different containers and environments for 10 months. The completely randomized design in a factorial 2 × 4 × 6 with four replications was used. The treatments were constituted of two storage environments (natural condition of laboratory (25 ± 2°C) and refrigerator (4 ± 2°C), combined with four types of packaging (PET bottle, plastic bag, burlap bag and kraft paper) and six storage periods (0, 2, 4, 6, 8, 10 months). The physiological seed quality was determined in a timeframe of two months by the following tests: Standard Test Germination - TPG, TPG first count, accelerated aging and electrical conductivity. It is concluded that the percentage of normal germination seedlings by TPG decreases linearly along the storage period; the PET bottle and Plastic bag preserved the vigor and viability of the seeds more efficiently along the storage, being that the PET bottle for being waterproof and tightly sealed has obtained better performance; and the refrigerator-controlled environment is the most suitable for storage of the pigeon pea among the tested environments.

Key words: *Cajanus cajan* L., seed quality, physiological deterioration, preservation, packaging.

INTRODUCTION

Cajanus cajan is the species in which pigeon pea belongs, still with a controversial origin between the African and Asian continents (Odeny, 2007). This culture can be found all over tropical countries for being easily adaptable to several soil and climatic conditions (Azevedo et al., 2007). In India, the largest producer of this fabacea accounting for 90% of production is an important food in many other tropical regions, including Asia, Africa, Central America and the Caribbean and Latin America (Torres et al., 2007; Mula and Saxena,

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2010). Pigeon pea is grown in 5 million hectares, being the sixth most important legume in the world (Varshney et al., 2012).

The pigeon pea is capable of producing abundant harvests of seeds rich in protein with values around 21 to 25% (Mula et al., 2010), even in low fertility soils, being adapted to high temperatures and drought conditions (Akande et al., 2010). Pigeon pea can survive quite well in degraded soils and tolerate water stress (Odeny, 2007). Thus, this culture has a good potential for farming in the “Cerrado” region, especially for the latter season. Nevertheless, the cultivation area of pigeon pea in Brazil is practically stagnant, especially due to the lack of quality seeds offered to farmers. In this sense, the influence of the tropical climate in the problems of seed storage, with high temperatures and humidity in natural environment has contributed to getting a poor quality product (Alencar et al., 2009).

After the seeds reach physiological maturity, the storage starts immediately, even before the beginning of the harvest known as the storage field (Baudet, 2003). However, being hygroscopic, the seed presents considerable variant in amount of water, depending on the relative humidity of air, and because of this low water amount of the seed associated with low temperature storage and lower relative humidity environment are key factors for the viability maintenance in extended periods (Rubim et al., 2013).

Storage is set as an important stage in the seed production process. The preservation of seed quality during this phase, that is, from harvest to the time of its use is an essential aspect to be regarded in the production process, because the efforts spent in the production phase may not be as effective if the seed quality is not maintained, at least until the time of sowing (Oliveira et al., 1999). It is also observed that every seed must be carefully processed and preserved during the storage period, until the moment of its usage, to ensure the preservation of its physiological quality (Marcos Filho, 2005). However, it is noteworthy that storage in tropical regions is one of the greatest hindrances to the maintenance of physiological seed quality.

Numerous factors influence the maintenance of viability and vigor of seed during storage for instance: initial seed physiological quality, parent plant vigor, climatic conditions during maturation, mechanical damage, drying conditions, adequate water content, relative air humidity, storage temperature, the action of microorganisms and insects, types of packaging and storage duration (Carvalho and Nakagawa, 2000; Toledo et al., 2009).

The utilization of top quality seed lots constitutes one of the major responsible factors for the success of a crop. With the application of the tests, one arrives to more correct decisions about the quality of seeds. Meantime, it is difficult to establish a relationship between the results of germination or seed vigor tests in the laboratory and field emergency, due to the interaction with environmental conditions at sowing time (Krzyzanowski et al., 1999).

The pigeon pea seed contains a considerably amount of starch (<50%) and protein (>22%), being classified as protein-amyl from the medium to long run, due to the concentration of protein as compared to storing starchy seeds - corn, rice, wheat, etc. A clear example of this affirmative was obtained by Martins Filho et al. (2001) by evaluating the physiological quality of soybean seeds whose protein levels are greater than those in the pigeon pea - around 45% when it was observed that there had been a decrease in vigor and viability starting at the 120th day of storage and after 210 days which presented a void effect. This behavior can be attributed as shown by Peske et al. (2006), a deviation in the chemical composition of the seed, hence the metabolism of proteins promotes partial breaking of these same amino acids presenting within this process, changes in chemical composition during the deterioration generating difficulties in obtaining seeds with high capacity growth and vigor.

Various techniques are used for storage of seeds, for example, bulk, porous or permeable containers (fabric bags – cotton or jute, sackcloth, multifold paper, plastic or polypropylene tracing) in packages resistant to moisture intrusion or semi-permeable (thin plastic bag or polyethylene and multifold paper bag laminated with polyethylene - kraft paper), and impermeable packages or moisture proof or completely sealed (aluminum cans).

Peske et al. (2006) could have direct influence over the final physiological quality of the harvested seed. However, there is no consensus in the literature over this hypothesis because there is a case of positive response as in a study by Bilia et al. (1994) about the behavior of hybrid corn seeds stored in three different conditions: cold chamber, dry chamber and atmosphere condition packed into kraft paper bags for six months, and it was found that the dry chamber has favored the quality of corn seeds. Conversely, Azevedo et al. (2003) in an evaluation on the physiological quality of sesame seeds stored in atmosphere conditions in a laboratory environment not controlled in a dry chamber, and in paper bags, plastic bags, and metal containers, have not shown any significant differences as to vigor for different containers tested.

Currently, there is few information available for research about pigeon pea, especially those related to the production and quality of seeds (Pedroso et al., 1988; Giomo, 1999) being narrowed down those for the seed storage in our conditions Nakagawa et al. (2009), and even internationally Godoy and Souza (2004) and Asalmol and Zade (1998). Therefore, investigative work about storage techniques of pigeon pea seeds is nonexistent. That way, it is essential the search for these detailed information, making it possible for technicians and producers a better data bank about the aspects related to the cultivation of this fabacea, especially, those related to the correct procedures for conservation of top

seed physiological quality, since this is an extremely important factor for achieving high productivity. Furthermore, it emphasizes that the storage is set as a fundamental practice for the control of seed quality, due to the fact of being a method in which the viability of the seeds can be preserved and the vigor kept at a reasonable level in the period between sowing and harvest. This study aimed to evaluate the physiological quality of pigeon pea seeds stored for ten months in different environments and packaging in the climatic conditions of the Central region of Goiás State, Brazil.

MATERIALS AND METHODS

General information

The pigeon pea seeds produced in the 2010/2011 season were purchased directly from producing firms, so that the performance of the work could take place on the premises of UEG/UnUCET more precisely in the Laboratory of drying and storage plant products of the Agricultural Engineering Course. Midget pigeon pea seeds were used. The seed samples were homogenized to sort the seeds out from undesirable inert material.

Experimental design and treatments

A completely randomized design was used in a factorial 2 × 4 × 6 frame with four replications. The treatments were constituted of two storage environments (natural laboratory conditions - 25 ± 2°C and refrigerator - 4 ± 2°C), four types of packaging (PET bottle, plastic bag, burlap bag and kraft paper) submitted to 10 months of storage every two months ratings (0, 2, 4, 6, 8 and 10 months).

Establishment and management

Before seed packing, water content was determined according to the rule for the analysis of seeds (BRASIL, 2009), the standard greenhouse method where the seeds were submitted to drying at 105°C ± 3° = 221 F for 24 h with results expressed in percentage (BRASIL, 2009). The determination of water content has been repeated at each assessment time of the physiological seed quality in all storage conditions.

Evaluated characteristics

The evaluation of physiological seed quality coming from the different treatments was checked by the following tests: Test pattern of germination – TPG, first count of TPG, accelerated aging and electrical conductivity. Test pattern of germination was conducted with four replications where 50 seeds/replicants were placed over three sheets of germ test paper, moistened with equivalent to three times its original water weight, rolled up and put up in a germination chamber at a temperature of 30°C = 86 F. The evaluation was made on the 10th (tenth) day after the performance of the test. The percentage of normal seedlings was logged in BRASIL (2009). The first germination test - conducted joint with TPG and considering the percentage of normal seedlings present on the 5th (fifth) day after the start. For the aging acceleration - 100 seeds / repetitions were distributed on the surface of a wire mesh fixed inside a plastic box - gerbox containing 40 ml of water, maintained at 42°C = 107 F and 100% of relative humidity for 48 h in a germination chamber (Krzyzanowski et al., 1999). After this period, the seeds were subjected to the TPG previously described to determine the percentage of normal seedlings on the 5th (fifth) day after the assembly of the test. The electrical conductivity test was conducted in the cup system recommended by Krzyzanowski et al. (1999) in which 50 pre-weighted seeds / repetitions were placed in plastic cups containing 75 ml of deionized water, placed and kept into a germination chamber at a constant temperature of 25°C = 77 F for 24 h. The reading for the electrical conductivity was performed by a conductivity meter and the results expressed in µS /cm /g of seeds.

Statistical analysis

The data was subjected to analysis of variance and when pertinent were submitted to the Tukey test at 5% probability. Statistical analysis was performed with the computer program Sisvar 4.6 (Ferreira, 2011).

RESULTS AND DISCUSSION

Through, the analysis of variance from the data obtained in the Standard Germination Test, First Count of TPG, Accelerated Aging and Electrical Conductivity were observed that the environment (E) influenced only the variable electrical conductivity (P>0.05%). The packaging (P) influenced Accelerated Aging variable (P>0.05%) and electrical conductivity (P<0.01%). The period (PE) of storing influenced all the variables (P<0.01%). The double interactions E×P and PE×P significantly influenced the variable in Electrical conductivity (P<0.01%). But the double interaction E×P influenced in the Accelerated Aging and Electrical Conductivity variables (P <0.01%). Regarding the triple interaction E×P×PE, we have the influence under the variables in the first count and electrical conductivity (P<0.01%) (Table 1).

There was linear decrease in the percentage of pigeon pea seed germination during the ten month-period storage (Figure 1). This behavior was expected and confirms Villela and Peres (2004) when it demonstrates that the seed quality does not improve during storage except in some cases of seeds endowed with a dormancy phenomenon which was not observed in this study. Moreover, Marcos Filho (2005) explains that from the physiological maturity point, a process of seed deterioration starts in a progressive rate until the unfeasibility of the seed or embryo death.

Figure 2 shows that for the first count of seedling test along the ten month-period store in different packaging and environments, it was found that PET packaging followed by plastic packaging were those which obtained the highest percentage of normal seedlings in both tested environments. This behavior can be explained by the permeability to water vapor that the featured by the packages tested for the PET packaging is classified as an impermeable plus being hermetically sealed not allowing interactions with water vapor from the external

Table 1. Results of analysis of variance (average squares) test applied in pigeon pea seeds for different environments, packaging and storage period.

Cause of variation	G. L.	Average Square			
		Germination	First count	Acceleration of aging	Electrical conductivity
Environment (E)	1	3.685^{ns}	12.505^{ns}	0.117^{ns}	372.681*
Packing (P)	3	3.920^{ns}	14.410^{ns}	154.223*	271.855**
Period (PE)	5	204.220**	321.324**	336.073**	1350.367**
E x P	3	98.782^{ns}	129.329^{ns}	87.543^{ns}	635.375**
E x PE	5	47.703^{ns}	56.255^{ns}	509.321**	742.521**
P x PE	15	67.046^{ns}	69.306^{ns}	47.361^{ns}	327.744**
E x P x PE	15	73.708^{ns}	81.721**	44.642^{ns}	263.777**
Residue	144	41.882	41.177	44.668	77.453
C.V.(%)	-	9.60	9.72	11.07	11,45

G.L. Degrees of liberty; * Significant at 5% probability by F test; ** Significant at 1% by F test; ns Not Significant.

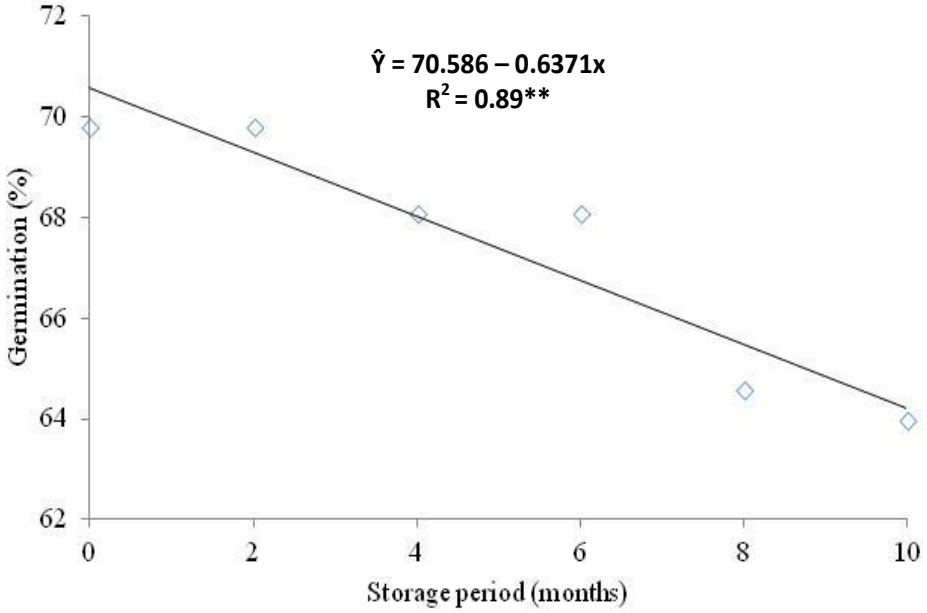

Figure 1. Percentage of normal seedling by TPG test applied in pigeon pea seeds during ten month-period storage.

environment. For Copeland and McDonald (1995), when seeds are stored in low temperature environments, they will probably acquire moisture due to the high relative humidity in these locations. Therefore, the use of impermeable packaging prevents the increase in moisture and deterioration rate.

The plastic container is characterized by being semi-permeable, since it only restricts the exchanges with the environment. To Stubsgaard (1992), plastic packaging is the most sensible for seed storing.

For PET and plastic packaging, the uncontrolled laboratory environment presented slightly higher averages than the controlled refrigerator environment. The lowest averages observed are from the kraft packaging and sackcloth respectively in both environments. It is noteworthy that the refrigerator controlled environment has obtained higher average values related to the ones in environment non-controlled Natural, concerning the packaging kraft and sackcloth. This phenomenon can be explained by the fact that these packs allow water vapor exchange with the environment, when the natural environment is subjected to greater fluctuations in temperature and relative humidity, speeding up the process of deterioration. According to Fowler (2000), packs that allow the exchange of moisture are recommended for seed storage for a short term or for

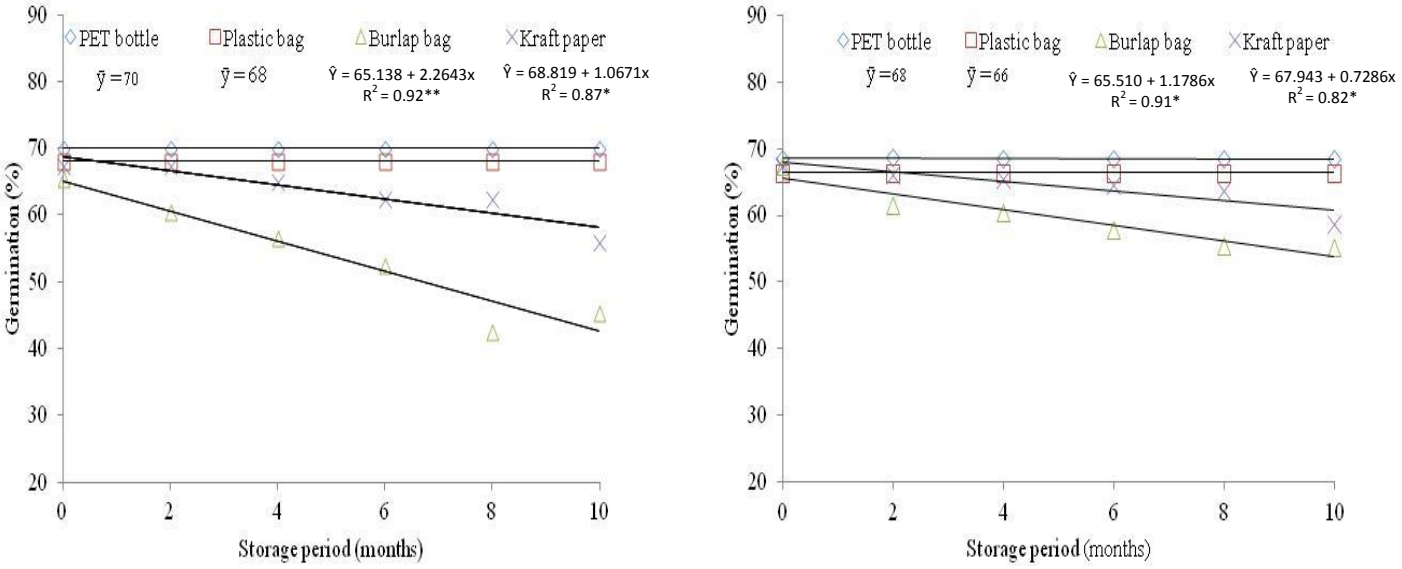

Figure 2. Percentage of germination of normal seedlings at the first count test applied for pigeon pea seeds during 10 months of storage in uncontrolled environment – laboratory (a) and controlled - refrigerator (b).

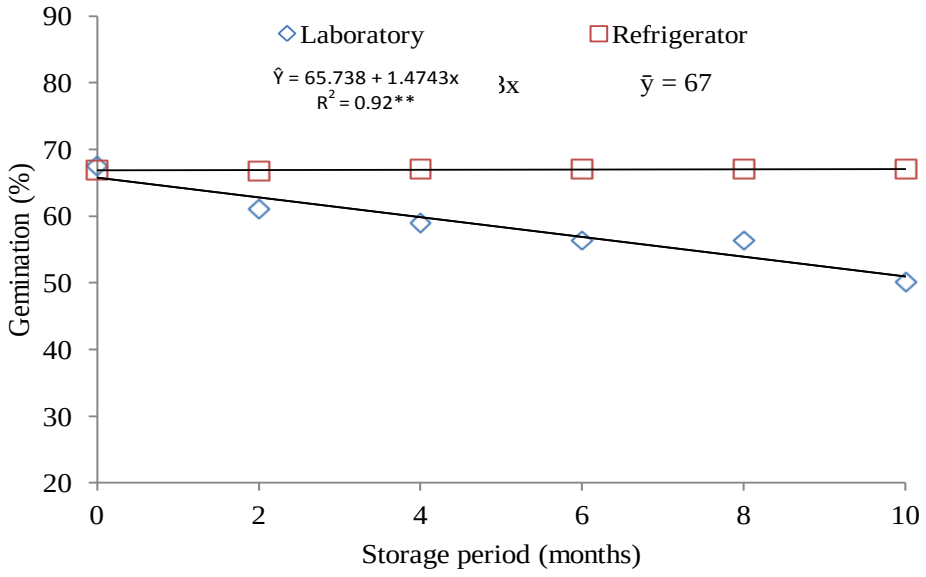

Figure 3. Percentage of germination for normal seedlings by accelerated aging test applied in pigeon pea seeds during 10 months of storage in different storage environments.

orthodox humid seeds. The moisture content of the seeds in this type of packaging varies with change in humidity.

Generally speaking, the percentage of germination of normal seedlings in PET and plastic packs for the uncontrolled environment and controlled-natural-refrigerator, maintained a steady linear behavior along the storage period with higher average values for the uncontrolled natural environment. The remaining packs kraft and sackcloth had a decreasing linear behavior in both environments with smaller percentage values for uncontrolled natural environment.

Besides the intrinsic characteristics of the seeds, the packaging, the environments and the storage period, there was the interference of external factors such as the attack from two pests from the Coleopteran species and two distinct species: The beetle (*Lasioderma cerealella*) and caterpillar (*Euphestia*). These two pests damaged the membrane, embryo and seed coat of the seed stored in kraft packs and sackcloth in the non-controlled natural environmental from the 8th month of storage.

Figure 3 compares the performance of the environments from the results of the first count of

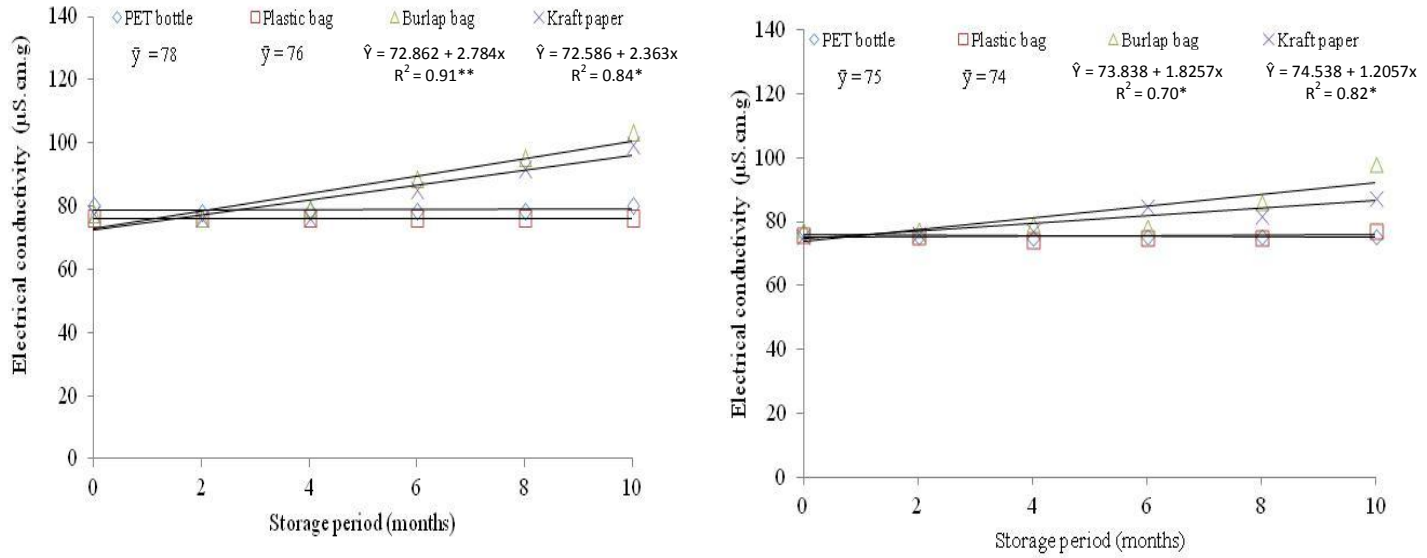

Figure 4. Reading of the electrical conductivity test applied for pigeon pea seeds during 10 months of storage in an uncontrolled laboratory environment - (a) and refrigerator controlled (b).

germination test of accelerated aging. In this test of vigor, it is obvious the superiority of the refrigerator controlled environment related to uncontrolled Natural environment, along the storage period. This can be explained because refrigerator environment maintains a low and constant temperature, contributing to a decrease in cellular metabolism of seeds, whereas the natural uncontrolled environment is subjected to oncoming changes in the external environment. Generally speaking, the lower the temperature and moisture content, the longer is the seed viability with some exceptions (Schmidt, 2007).

The results shown in Figure 4 in relation to the electrical conductivity test applied to pigeon pea seeds for ten months of storage in different packaging and environments, and demonstrate that burlap and kraft packs in both environments show an increase of µS/cm/g higher than other containers over the time, however, it is worth highlighting the results of the non-controlled natural environment because they are superior to the refrigerator controlled. Regarding PET and plastic packing, slightly higher values were observed for PET-packaging in a non-controlled natural environment. As for the refrigerator controlled environment both packing showed similar values during storage.

Moreover, the uncontrolled natural environment obtained higher readings than the refrigerator controlled environment for all packaging studied over the period of storage for the electrical conductivity test. This behavior can be attributed to the fact that the seeds stored under uncontrolled natural environment have suffered greater influence of the external environment, causing acceleration to its deterioration process. The readings of electrical conductivity in both storage environments grow linearly over time for packaging that allow greater exchange with the environment (burlap and kraft), demonstrating that there was a progressive increase in the breakdown and loss of integrity of the cell membrane system seed along storage time.

The packaging impermeable and semi-permeable, PET and plastic respectively, maintained a good overall quality of the seed cell membrane system during the period of storage.

Conclusion

The percentage of normal seedling germination in the TPG decreases linearly along the storage period. The packaging PET and plastic maintained the vigor and viability for the pigeon pea seeds over the ten-month storage period, independent of the tested environments. The vigor and viability of pigeon pea seeds were better maintained over the ten months of storage for impermeable and hermetically sealed packaging PET. The refrigerator controlled environment is the fittest for the storage of pigeon pea among the tested environments.

Conflict of Interest

The authors have not declared any conflict of interest.

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REFERENCES

Akande KE, Abubakar MM, Adegbola TA, Bogoro SE, Doma UD (2010). Chemical evaluation of the nutritive quality of pigeon pea (*Cajanus cajan* (L.) Millsp.). Int. J. Poult. Sci. 9(1):63-65. http://dx.doi.org/10.3923/ijps.2010.63.65

Alencar ER, Faroni LRD, Lacerda Filho AF, Peternelli LA, Costa AR (2009). Quality of soybean grains stored under different conditions. Rev. Bras. Eng. Agríc. Ambient. 13(5):606-613. http://www.scielo.br/pdf/rbeaa/v13n5/v13n05a14.pdf

Asalmol MN, Zade VR (1998). Effect of seed treatment on storability of seeds of different crops. Seed Res. 26(1):53-56.

Azevedo MRQA, Gouveia JPG, Trovão DMM, Queiroga VP (2003). Influence of packing and storage conditions on the vigor of sesame seeds. Rev. Bras. Eng. Agríc. Ambient. 7(3):519-524. http://dx.doi.org/10.1590/S1415-43662003000300019

Azevedo RL, Ribeiro GT, Azevedo CLL (2007). Feijão guandu: uma planta multiuso. Revista da Fapese 3(2):81-86.

Baudet L (2003). Armazenamento de sementes. In: Peske ST, Rosenthal MD, Rota GM (eds). Sementes: fundamentos científicos e tecnológicos. Pelotas: Gráfica Universitária-UFPel, pp. 369-418.

Bilia DAC, Fancelli AL, Marcos Filho J (1994). Behaviour of hybrid corn seeds during storage under different conditions of air temperature and relative humidity. Sci. Agric. 51(1):153-154. http://dx.doi.org/10.1590/S0103-90161994000100022.

BRASIL (2009). Regras para análises de sementes. Ministério da Agricultura e relativa Reforma Agrária. Brasília. P. 395.

Carvalho NM, Nakagawa J (2000). Sementes: ciência, tecnologia e produção. 4. ed. Jaboticabal: FUNEP/UNESP. P. 588.

Copeland LO, McDonald MB (1995). Seed science and technology. 3. ed. New York: Chapman & Hall. P. 409.

Ferreira DF (2011). Sisvar: a computer statistical analysis system. Ciênc. Agrotec. 35(6): 1039-1042.

Fowler JAP (2000). Superação de dormência a armazenamento de sementes de espécies florestais. In: Galvão APM (Org.). Reflorestamento de propriedades rurais para fins produtivos e ambientais: um guia para ações municipais e regionais. Brasília: Embrapa Comunicação para Transferência de Tecnologia; Colombo, PR: Embrapa Florestas, pp. 77-100.

Giomo GS (1999). Efeitos de espaçamento no crescimento da planta, na produção e qualidade de sementes de guandu (*Cajanus cajan* (L.) Millsp), em semeadura tardia. Botucatu, P. 83. Dissertação (Mestrado em Agricultura) - Faculdade de Ciências Agronômicas, Universidade Estadual Paulista.

Godoy R, Souza, FHD (2004). Dormancy on pigeon pea seeds (*Cajanus cajan* (L.) Millsp). R. Bras. Zootec. 33:2201-2205.

Krzyzanowski FC, Vieira RD, França Neto JB (1999). Vigor de sementes: conceitos e testes. Londrina: ABRATES. P. 218.

Marcos Filho J (2005). Fisiologia de sementes de plantas cultivadas. Piracicaba: Fealq. P. 495.

Martins Filho S, Lopes JC, Rangel OJP, Tagliafferre C (2001). Physiological seed quality evaluation of soybean during storage in uncontrolled warehouse conditions in Alegre, in the State of Espirito Santo. Rev. Bras. Sementes 23:201-208. http://www.abrates.org.br/revista/artigos/2001/v23n2/artigo28.pdf

Mula MG, Saxena KB (2010). Lifting the level of awareness on pigeon pea—a global perspective. International Crops Research Institute for the Semi-Arid Tropics.

Nakagawa J, Claudio C, Toledo MZ (2009). Germination of stored pigeon pea seeds. Rev. Bras. Sementes 31:43-48. http://dx.doi.org/10.1590/S0101-31222009000400005

Odeny DA (2007). The potential of pigeon pea (*Cajanus cajan* (L.) Millsp.) in African. Natural Resources Forum 31:297–305. http://dx.doi.org/10.1111/j.1477-8947.2007.00157.x

Oliveira JA, Carvalho MLM, Vieira MGGC, Von Pinho EVR (1999). Comportamento de sementes de milho colhidas por diferentes métodos, sob condições de armazém convencional. Ciênc. Agrotec. 23:289-302.

Pedroso PAC, Vieira RD, Sader R, Scotton LA (1988). Effect of plant spacing and density on seed production and quality. Rev. Bras. Sementes 10:45-53. http://www.abrates.org.br/revista/artigos/1988/v10n2/artigo04.pdf

Peske ST, Lucca Filho AO, Barros ACSA (2006). Sementes: fundamentos científicos e tecnológicos. 2. ed. Pelotas: Ed. Universitária UFPel. P. 470.

Rubim RF, Freitas SP, Vieira HD, Gravina GA (2013). Physiological quality of fennel (*Foeniculum vulgare* Miller) seeds stored in different containers and environmental conditions. J. Seed Sci. 35(3):331-339. http://dx.doi.org/10.1590/S2317-153720130003000009

Schmidt L (2007). Tropical forest seed. New York: Springer. P. 409.

Stubsgaard F (1992). Seed storage. n.c-9, Dinamarca: Danilda Forest Seed Centre.

Toledo MZ, Fonseca NR, César ML, Soratto RP, Cavariani C, Crusciol CAC (2009). Physiological quality and storage of bean seeds as affected by late side dressing nitrogen. Pesqui. Agropecu. Trop. 39:124-133.

Torres A, Frias J, Granito M, Vidal-Valverde C (2007). Germinated *Cajanus cajan* seeds as ingredients in pasta products: Chemical, biological and sensory evaluation. Food Chem. 101(2):202–211. http://dx.doi.org/10.1016/j.foodchem.2006.01.018

Varshney RK, Chen W, Li Y, Bharti AK, Saxena RK, Schlueter JA, Donoghue MTA, Azam S, Fan G, Whaley AM, Farmer AD, Sheridan J, Iwata A, Tuteja R, Penmetsa V, Wu W, Upadhyaya HD, Yang SP, Shah T, Saxena KB, Michael T, Mccombie WR, Yang B, Zhang G, Yang H, Wang J, Spillane C, Cook DR, May GD, Xu X, Jackson SA (2012). Draft genome sequence of pigeon pea (*Cajanus cajan*), an orphan legume crop of resource-poor farmers. Nat. Biotechnol. 30(1):83-89. http://dx.doi.org/10.1038/nbt.2022

Villela FA, Peres WB (2004). Coleta, beneficiamento e armazenamento. In: Ferreira A. In: Ferreira AG, Borghetti F. (Ed.). Germinação: do básico ao aplicado. Porto Alegre: Artmed, pp. 149-162.

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Phosphorus fertilization associated to inoculation of maize with diazotrophic bacteria

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The effects of diazotrophic bacteria inoculation associated to phosphate fertilization on plant growth and leaf gas exchange parameters in maize plants (*Zea mays* L.) were investigated in the present study. Maize plants were grown in 13-L pots filled with clayey Rhodic Hapludox in a greenhouse. Treatments were arranged in a randomized block design in a 4 × 2 factorial: four seed inoculation treatments [control (non-inoculated); inoculation with *Azospirillum brasilense* strain AbV5; inoculation with *Herbaspirillum seropedicae* strain SmR1; and inoculation with two bacteria strains (*A. brasiliense* + *H. seropedicae*)] and two phosphate fertilization levels [no fertilized or fertilized with phosphorus (300 mg dm^{-3} of P_2O_5)]. Phosphorus fertilization resulted in higher plant height, stem diameter, number of leaves per plant, leaf area, dry matter yield of leaves, stems and sheaths of maize plants, regardless of seed inoculation with diazotrophic bacteria. Seed inoculation with *A. brasilense* and *H. seropedicae* increased in 42% of the volume root, in 52% of the root dry matter and 25% of the plant height of maize, indicating an increase in the phosphorus solubilization or higher phosphorus use provided by the maize root system. Seed inoculation with *A. brasilense* associated to phosphorus fertilization increased in 23% of the relative chlorophyll content, resulting in higher metabolic structure to the photosynthetic activity of maize plants. The leaf CO_2 assimilation rate was not affected by the phosphorus fertilization and maize seed inoculation with *A. brasilense* and *H. seropedicae*.

Key words: Plant growth promoting bacteria, phosphate solubilization, gas exchange, *Azospirillum brasilense*, *Herbaspirillum seropedicae*, *Zea mays*.

INTRODUCTION

The maize culture (*Zea mays* L.), is the grain species most cultivated in Brazil and in the world, followed by the wheat culture (USDA, 2014; IBGE, 2014). The Brazilian production reached 85.5 million tons of grain, with grain

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yield of 5.1 ton ha^{-1} in 15.8 million of hectares (CONAB, 2014) and 968.8 million tons in the world (USDA, 2014). In Brazil, the study of biological nitrogen fixation (BNF) in grasses was initiated by Döbereiner (1953), detecting Azotobacter in acid soil of "Baixada Fluminense". Thereafter, the studies were extended with associative bacteria in sugar cane (Döbereiner and Ruschel, 1958) and *Paspalum notatum* cv. Batatais (Döbereiner, 1966).

Initially, these ones were called diazotrophic bacteria (DB) due the acting in the conversion of atmospheric nitrogen (N) in assimilable N to plants (Döbereiner and De-Polli, 1980), and can act with plant growth promoting bacteria (PGPB) (Strzelczyk et al., 1994; Carvalho et al., 2009). Indeed, there are studies relating benefits in the accumulation of dry weight of leaves (Rodrigues et al., 2014), shoot growth and increased of 7% in grain yield (Dartora et al., 2013), obtained through the inoculation with AbV5 strain of *Azospirillum brasilense* in maize culture. The improvement of these agronomic parameters are related to auxin, gibberellin (Strzelczyk et al., 1994) and ethylene production by bacteria; (Strzelczy and Pokojska-Burdziej, 1984). Recently, the solubilization ability of phosphate in soil by DB e PGPB has been demonstrated (Jain et al., 2010; Baldotto et al., 2012; Walpola and Yoon, 2013).

And to ensure the maximum genotypic production of a maize plant, high dosage of phosphorus fertilization is required in addition to nitrogen fertilization divided in three applications from sowing to phenological stage stadium V_8 (Coelho et al., 1995). In that sense, with several benefits in plants proportionated by associative bacteria, the use of agricultural inputs as principal source of nitrogen and phosphorus, can be reduced.

The phosphorus (P) is macronutrients that have important compounds in the active photosynthetic cells, directly influencing the gas exchange of plants, in both the photosynthesis and cellular respiration process. The photosynthesis is carried out through chlorophyll molecules contained in organelles called chloroplasts, which are activated by luminous energy, transforming it in chemical energy, stored in phosphate compounds such as adenosine triphosphate (ATP) (Taiz and Zeiger, 2013). During the night this action does not occur, however, it occurs only during breath, the reverse process where the plants release CO_2 and consume oxygen, consuming ATP (Taiz and Zeiger, 2013).

The gas exchange by maize plants are carried out in the mesophyll of leaves through stomatal openings. It is possible due to the difference of water and CO_2 concentration between the internal and external environment of leaves. At the higher water concentration gradient than CO_2 gradient, it results in higher water flow than CO_2 in the stomata (Farquhar and Raschke, 1978). In that sense, the interference begins in stomatal conductance (g_s), affecting the transpiration (E) and net CO_2 assimilation rate (A) (Cowan and Troughton, 1971).

In view of the above, the aim of this study was to evaluate the influence of inoculation with *A. brasilense* AbV5 and *Herbaspirillum seropedicae* SmR1 species in maize culture, conducted with and without phosphorus fertilization.

MATERIALS AND METHODS

An experiment was conducted in a greenhouse in Marechal Cândido Rondon, Paraná State, Brazil (24° 46' S, 54° 22' W, and altitude of 400 m), in 13-L plastic pots. The soil was a Rhodic Hapludox (Red Latosol in the Brazilian classification) with 720 g kg^{-1} of clay, 110 g kg^{-1} of silt, and 170 g kg^{-1} of sand. Samples were taken from the surface layer (0 to 0.20 m), air dried, sieved through a 2 mm mesh, and analyzed as in Embrapa (2009). Soil chemical analysis showed pH ($CaCl_2$ 0.01 mol L^{-1}): 4.8, 12.3 g dm^{-3} of organic matter, 32 mg dm^{-3} of P (Mehlich-1), 3.87 $cmol_c$ dm^{-3} of H + Al, 0.58 $cmol_c$ dm^{-3} of K, 2.45 $cmol_c$ dm^{-3} of Ca, 0.95 $cmol_c$ dm^{-3} of Mg, 7.85 $cmol_c$ dm^{-3} of CEC, 51% of soil base saturation, 4.10 mg dm^{-3} of Cu, 3.00 mg dm^{-3} of Zn, 45.0 mg dm^{-3} of Mn and 59.1 mg dm^{-3} of Fe.

The experiment was arranged in a randomized block design, using four treatments for the seed inoculation factor [control (non-inoculated); inoculation with *A. brasilense* strain AbV5; inoculation with *H. seropedicae* strain SmR1; and inoculation with two bacteria strains (*A. brasiliense* + *H. seropedicae*)] and two levels for the phosphorus fertilization factor [no fertilized or fertilized with phosphorus (300 mg dm^{-3} of P_2O_5)], considering a factorial arrangement (4 × 2). A total of 32 pots were used – four pots per treatment. Maize seeds were inoculated with 4.0 mL of inoculant for each thousand seeds and then maintained at rest for twelve hours in the shade. Inoculants were provided by the Biochemistry and Molecular Biology Laboratory, Federal University of Paraná (UFPR), Curitiba, Brazil and had concentration of 2×10^7 CFU mL^{-1}. The P source used was simple superphosphate (18% P_2O_5; 25% CaO and 12% S). The basic fertilization was carried out with applying 30 mg dm^{-3} of N as urea (45% N), 100 mg dm^{-3} of K as potassium chloride (60% K_2O) and 40 mg dm^{-3} of S as gypsum (13% S and 18% Ca). The fertilizer amount applied was performed according to the recommendations for greenhouse crops as described by Alvarez and Fonseca (1990).

Six seeds of maize (*Z. mays* L., PIONEER 30F53H hybrid) were sown in pots, and four days after seedling emergence, they were thinned to three plants per pot. The soil water content was maintained near at the field capacity with two daily irrigations.

During the growth and development of maize plants were measured using biometric variables. Maize plant height (from the soil surface to the apex of the plants) was measured at 7, 14, 21, 41 and 52 days after sowing (DAS). Chlorophyll readings were made using a SPAD meter (SPAD 502® Minolta) at 21 and 52 DAS, on the third fully expanded leaf from the apex of the three plants in each pot. The stem diameter (mm) and number of leaves per plant were measured only at 52 DAS.

Leaf gas exchange was monitored with an infrared gas analyzer (LI-6400XT, LICOR, Inc. USA), in the maize growing stage of eight developed leaves – V5 (50 DAS). Measurements of leaf CO_2 assimilation rate (A, in µmol CO_2 m^{-2} s^{-1}), transpiration rate (E, in mmol H_2O m^{-2} s^{-1}), stomatal conductance (g_S, mol m^{-2} s^{-1}) and intercellular CO_2 concentration (Ci, in µmol mol^{-1}) were taken at 10:00 and 22:00 h, allowing estimation of leaf respiration rate of maize plants. Water-use efficiency – WUE (A/E) was also calculated. At 10:00 h (10:00 am), the gas exchange parameters were determined at 400 µmol mol^{-1} of [CO_2] and 1500 µmol m^{-2} s^{-1} of photosynthetic photon flux density (PPFD), with standard deviation of 0.7444. The mean photons density of the external environment provided by the apparatus was 716.35 µmol m^{-2} s^{-1}, 48.97% relative humidity, and air flow of 499.43 mL per minute. In the evaluation at 22:00 h (10:00 pm), the gas exchange parameters

Table 1. Analysis of variance for plant height at 7, 14, 21, 41 and 52 days after sowing (DAS), plant hybrid corn seed via 30F53H inoculated with plant growth promoting bacteria as a function of phosphate fertilizer[1].

Source of variation	Mean square				
	Height 7 DAS	Height 14 DAS	Height 21 DAS	Height 41 DAS	Height 52 DAS
P fertilization	0.38^{ns}	34.05**	869.65**	7310.52**	5447.07*
Inoculation	0.20^{ns}	3.84^{ns}	0.31^{ns}	128.01^{ns}	275.41^{ns}
Interaction	0.91^{ns}	2.73^{ns}	143.81**	524.16^{ns}	360.98^{ns}
Residual	0.32	2.61	19.16	414.03	689.35
C.V. (%)	6.46	7.58	15.87	32.98	28.89
Average	8.79	21.34	27.59	61.70	91.48

[1] * = P ≤ 0.05; ** = P ≤ 0.01; ns = Not significant, by Tukey's test Fisher-Snedecor.

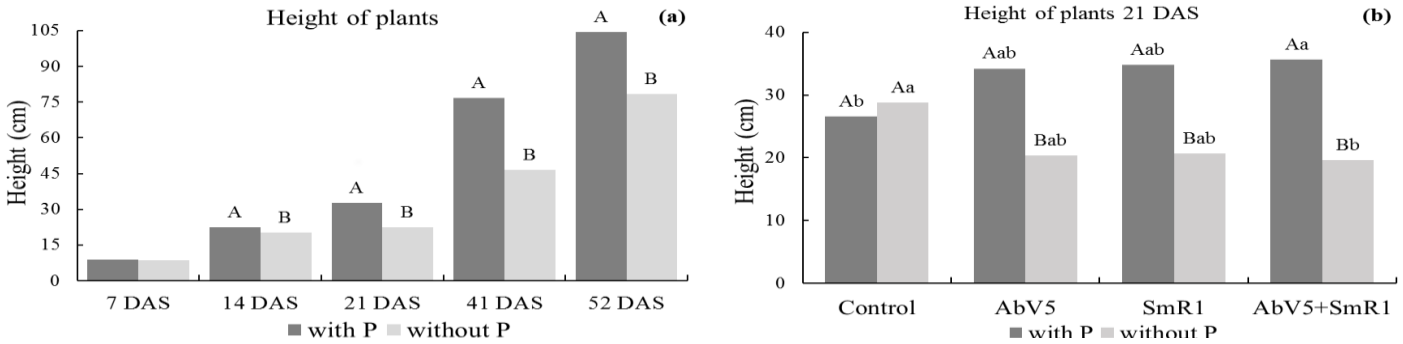

Figure 1. Height of plants at 7, 14, 21, 41, and 52 days after sowing (DAS) the average of the inoculation treatments (a)[1] and height of plants inoculated with control treatment, *Azospirillum brasilense*, *Herbaspirillum seropedicae* and association of two species at 21 DAS (b)[2], depending on the phosphate fertilizer[1]. [1]Means followed by different letters UPPERCASE comparing phosphate fertilizer in plant height, differ significantly by the Tukey test at 5%; [2]Means followed by the same letter LOWERCASE comparing phosphorus fertilization in seed inoculation and seed inoculation UPPERCASE comparing within phosphate levels do not differ significantly by the Tukey test at 5%.

were measured in dark room (no photons) with 400 µmol mol^{-1} of [CO_2], 44.95% relative humidity, and air flow of 499.39 mL per minute. At 52 days after sowing, the plants in all treatments were harvested and separated into roots, stems, sheaths and leaves. The plant parts were dried in air-forced oven at 65°C for three days, and then weighed.

The leaf area (LA, dm^2/pot) was determined using the following equation proposed by Benincasa (2003):

$$LA = [(LAs \times TDML)/DMs]$$

where LAs is the leaf area of the sample collected, TDML is the total dry matter of leaf and DMs is the dry matter of the sample collected. Root volume (RV, cm^3 pot^{-1}) was determined by water displacement using a calibrated cylinder.

Data were analyzed by ANOVA, and the means of phosphorus application and seed inoculation treatments were compared by F test and Tukey test, respectively, both at the 0.05 level of confidence. All analyses were performed using Sisvar 5.1 software for Windows (Statistical Analysis Software, UFLA, Lavras, MG, BRA) (Ferreira, 2011).

RESULTS AND DISCUSSION

Plant height at 7 days after sowing (DAS) was not affected by phosphate fertilization and inoculation of maize seeds with diazotrophic bacteria (Table 1). This absence of plant height response to phosphorus fertilization and diazotrophic bacteria inoculation was due to the nutrients needed for seedling which are being supplied by the proviso contained in the seeds (Marcos Filho, 2005). At 7, 14, 21, 41 and 52 DAS, the highest maize plant height was obtained in the treatment with phosphorus fertilization, regardless of seed inoculation with diazotrophic bacteria (Figure 1a).

At 21 DAS there was significant interaction between phosphorus fertilization and inoculation of maize seeds with diazotrophic bacteria. The phosphorus fertilization associated to inoculation of maize with *A. brasilense* and *H. seropedicae* resulted in higher plant height compared to the control treatment. Inoculation of *A. brasilense* and *H. seropedicae* isolated or associated showed similar maize height. However, when there was no phosphorus application, maize response was different (Figure 1b). When maize plants were not fertilized with phosphorus, the diazotrophic bacteria provided lower values of plant height at 21 DAS, and associated inoculation of *A. brasilense* and *H. seropedicae* provided maize plants of

Table 2. Analysis of variance for stem diameter (SD), root volume (RV), number of leaves (NL), leaf area (LA), relative chlorophyll content (SPAD) at 21 and 52 days after sowing (DAS), leaf dry weight (LDW), stem+sheaths dry weight (SSDW) and roots (RDW) of hybrid corn plants via seed 30F53H inoculated with plant growth promoting bacteria as a function of phosphate fertilizer[1].

Source of variation	Mean square								
	SD	RV	NL	LA	SPAD 21	SPAD 52	LDW	SSDW	RDW
P fertilization	269.17**	105800.00**	4.48*	91.91**	131.62*	18.76^{ns}	187.49**	307.36**	130.17**
Inoculation	3.31^{ns}	2937.50^{ns}	1.29^{ns}	1.24^{ns}	4.91^{ns}	28.83^{ns}	2.18^{ns}	36.74^{ns}	3.29^{ns}
Interaction	41.98^{ns}	20708.33**	1.13^{ns}	9.39^{ns}	99.12*	33.06^{ns}	21.52^{ns}	6.51^{ns}	30.79**
Residual	18.05	3252.98	1.57	5.91	26.51	11.56	10.08	21.68	4.4
C.V. (%)	29.32	36.95	17.96	46.16	17.64	10.87	46.97	62.75	43.25
Average	14.49	154.37	6.99	5.27	29.19	31.28	6.76	7.41	4.85

[1] * = P ≤ 0.05; ** = P ≤ 0.01; ns = Not significant, by Tukey's test Fisher-Snedecor.

smaller stature compared to the uninoculated control. The association of *A. brasilense* (a facultative endophytic bacteria) and *H. seropedicae* (an obligate endophytic bacteria) inoculation (Baldani and Baldani, 2005), have direct influence on the height of maize plants. The diazotrophic bacteria may have used the soil available P as support for its maintenance and development, favoring the associative characteristic to plants, in this case, maize under P fertilization.

Furthermore, several bacterial strains, particularly those belonging to the genera *Azospirillum*, *Bacillus*, *Pseudomonas* and *Rhizobium* have been described and investigated in detail for their phosphate-solubilizing capabilities from the organic and inorganic soil pools (Hameeda et al., 2008; Souchie et al., 2006; Murty and Ladha, 1988). Considering that P availability is a limiting step in plant nutrition, this evidence suggests a fundamental contribution of phosphate-solubilizing bacteria to plant nutrition and, therefore, to the improvement of plant growth performance. Inoculation of rice (*Oryza sativa* L.) seeds with *Azospirillum lipoferum* strain 34H increased the phosphate ion content and resulted in significant improvement of root length and shoot dry matter (Murty and Ladha, 1988). Hameeda et al. (2008) verified that the seed inoculation with *Pseudomonas niger* strain CDB 35 (an isolate with high P solubilization potential) increased the grain yield of maize by 64% compared to the uninoculated control. Strains of phosphate solubilizing bacteria promoted increased P uptake by the mung bean plants [*Vigna radiata* (L.) Wilczek], improving the performance of plant growth (Walpola and Yoon, 2013).

Phosphorus fertilization significantly affected the stem diameter, number of leaves per plant, leaf area, leaf dry matter and stem plus leaf sheath dry matter. The seed inoculation with diazotrophic bacteria did not affect these variables (Table 2). For relative chlorophyll content measured through SPAD readings at 21 DAS, root volume and root dry matter, there was significant interaction between phosphorus fertilization and inoculation of maize seeds with diazotrophic bacteria.

Phosphorus fertilization increased the number of leaves per plant, stem diameter, leaf area, leaf dry matter and stem plus leaf sheath dry matter of maize plants by 12, 33, 49, 53 and 59%, respectively, compared to the treatment without P fertilization (Table 3). Phosphorus and nitrogen are important macronutrients for growth and development of plants. Phosphorus is a component of the complex nucleic acid structure of plants, which regulates protein synthesis. This element is also associated with complex energy transformations in the plant and components of cell membranes. Phosphorus is, therefore, important in cell division and development of new tissue (Taiz and Zeiger, 2013). These functions of P in plants justifies the positive results in the biometric variables, observed in this study (Table 2).

With the addition of phosphate fertilizer, the inoculation with the combination of the two bacteria strains (*A. brasilense* and *H. seropedicae*) showed higher root volume (247.5 cm^3 pot^{-1}) compared to the uninoculated control (142.5 cm^3 pot^{-1}), but no significant difference (Figure 2a). In the absence of phosphate fertilizer, the inoculation of *H. seropedicae* resulted in smaller root volume compared to the uninoculated control. The inoculation with two bacteria strains (*A. brasilense* and *H. seropedicae*) associated to phosphorus fertilization provided higher root volume compared to the inoculation in the absence of phosphate fertilizer. Phosphorus deficiency can lead to nutritional imbalance of plants, such as in the Ca/P ratio (Novais et al., 2007). This nutritional imbalance could affect the composition of cell wall and tissues and therefore the growth and development of maize plants.

Inoculation with combination of the two bacteria strains (*A. brasilense* and *H. seropedicae*) as affected by phosphorus fertilization showed higher root dry matter compared to the uninoculated control (Figure 2b). In turn, when there was no addition of phosphate fertilizer, inoculation of the two bacteria strains resulted in less root dry matter compared to the uninoculated control. These data indicate that the phosphorus fertilization favored diazotrophic bacteria inoculation in relation to the

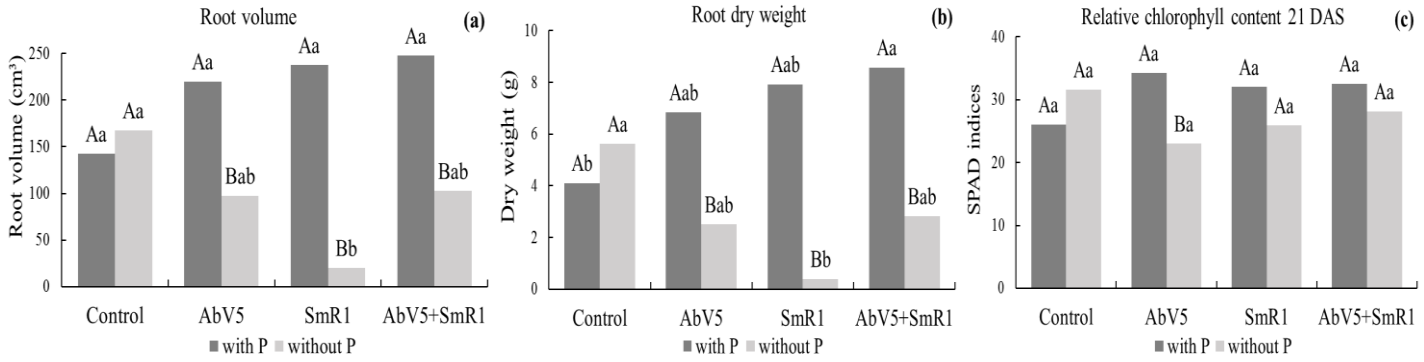

Figure 2. Root volume (a), root dry weight (b) and relative chlorophyll content (c) 21 days after sowing maize hybrid seed via 30F53H inoculated with plant growth promoting bacteria as a function of phosphate fertilizer[1]. [1] Means followed by the same letter UPPERCASE comparing phosphorus fertilization in seed inoculation, LOWERCASE comparing seed inoculation in phosphate fertilizer, not differ significantly by the Tukey test at 5%.

Table 3. Stem diameter (SD), number of leaves (NL), leaf area (LA), leaf dry weight (LDW) and stem+sheaths dry weight (SSDW) of maize plants inoculated via 30F53H hybrid seed with plant growth promoting bacteria as a function of phosphate fertilizer[1].

P	SD (mm)	NL	LA (dm²)	LDW (g)	SSDW (g)
With	17.39[a]	7.46[a]	6.96[a]	9.18[a]	10.52[a]
Without	11.59[b]	6.52[b]	3.57[b]	4.34[b]	4.32[b]
Average	14.49**	6.99*	5.27**	6.75**	7.42**
C.V. (%)	29.32	17.96	46.16	46.97	62.75
L.S.D.	3.12	0.92	1.79	2.33	3.42

[1] Means followed by the same lowercase letters (column) do not differ by Tukey's test ($p > 0.05$). * = $P \leq 0.05$; ** = $P \leq 0.01$; ns = Not significant, by Tukey's test Fisher-Snedecor.

development of the maize root system, especially when the two strains were inoculated in combination. This result indicates that the phosphate solubilization promoted by diazotrophic bacteria (Jain et al., 2010) can improve the use efficiency of this nutrient.

Dartora et al. (2013) evaluated the effects of diazotrophic bacteria inoculation associated with nitrogen fertilization, and found positive influence when maize plants were inoculated with *A. brasilense* and *H. seropedicae*, resulting in increased stem diameter, shoot dry matter and grain yield of up to 7% compared to the uninoculated control. Similarly, Lana et al. (2012) observed increase in maize grain yield from 7 to 15%, using the seed inoculation with *A. brasilense*. The genera *Azospirillum* and *Herbaspirillum*, due to the preference of colonization in local distinct of the plants, and the inoculation of two strains (*A. brasilense* and *H. seropedicae*) may have favored the nutrient assimilation in the presence of available P, improving the translocation and distribution of nutrients in the plant, and consequently the maize development.

The relative chlorophyll content in the maize leaves measured through SPAD readings at 21 DAS, showed interaction between the factors studied, differing only when there has been inoculation of *A. brasilense*, and the highest relative chlorophyll content was obtained when maize plants were fertilized with P (Figure 2c). The increase in the relative chlorophyll content to phosphate fertilization may be associated with the production of phytohormones due to the bacterial activity, such as auxin and gibberellin (Strzelczyk et al., 1994). In a recent study, Baldotto et al. (2012) found that maize plants inoculated with diazotrophic bacteria or phosphate solubilizing bacteria showed higher concentrations of nitrogen, phosphorus and potassium in the leaf tissue, resulting in the higher plant growth and increase of the SPAD index. The phosphorus fertilization and maize seed inoculation with *A. brasilense* and *H. seropedicae* did not affect the leaf gas exchange parameters related to photosynthesis and respiration of corn plants. There was effect of P fertilization only for the intercellular CO_2 concentration (Ci) and water-use efficiency (WUE) on the assessment performed during the photosynthetically active leaves (10:00 a.m.), and for the leaf CO_2 assimilation rate (*A*) in the period of plant respiration at 10:00 p.m. (Table 4 and Figure 3).

Table 4. Summary of analysis of variance performed for gas exchange during photosynthesis (10:00 a.m.) and respiration (10:00 p.m.) on maize leaves, rate of net CO_2 assimilation (*A*), leaf transpiration (*E*), stomatal conductance (g_s) and the water-use efficiency (*WUE*) from the leaves of plants inoculated via 30F53H hybrid corn seed with plant growth promoting bacteria as a function of phosphate fertilizer[1].

Source of variation	*A* (µmol CO_2 m^{-2} s^{-1})	g_s (mol m^{-2} s^{-1})	*E* (mmol H_2O m^{-2} s^{-1})	*Ci* (mmol m^{-2} s^{-1})	*WUE*
	Mean square (10:00 am)				
P fertilization	5.355^{ns}	0.003^{ns}	3.421^{ns}	11021.084*	1.164**
Inoculation	12.176^{ns}	0.004^{ns}	1.151^{ns}	1548.705^{ns}	0.162^{ns}
Interaction	28.292^{ns}	0.002^{ns}	0.747^{ns}	946.880^{ns}	0.197^{ns}
Residual	24.620	0.005	1.343	1487.735	0.110
C.V. (%)	18.59	44.46	20.47	85.39	7.00
Average	26.69	0.16	5.66	45.17	4.75
	Mean square (10:00 pm)				
P fertilization	1.371*	0.000^{ns}	0.022^{ns}	19589.874^{ns}	8.367^{ns}
Inoculation	0.063^{ns}	0.000^{ns}	0.004^{ns}	7739.199^{ns}	6.542^{ns}
Interaction	0.448^{ns}	0.000^{ns}	0.001^{ns}	25679.297^{ns}	7.256^{ns}
Residual	0.255	0.000	0.009	36059.995	21.318
C.V. (%)	18.52	37.52	35.42	21.08	42.33
Average	2.73	0.01	0.28	900.82	10.91

[1] * = P ≤ 0.05; ** = P ≤ 0.01; ns = Not significant, by Tukey's test Fisher-Snedecor.

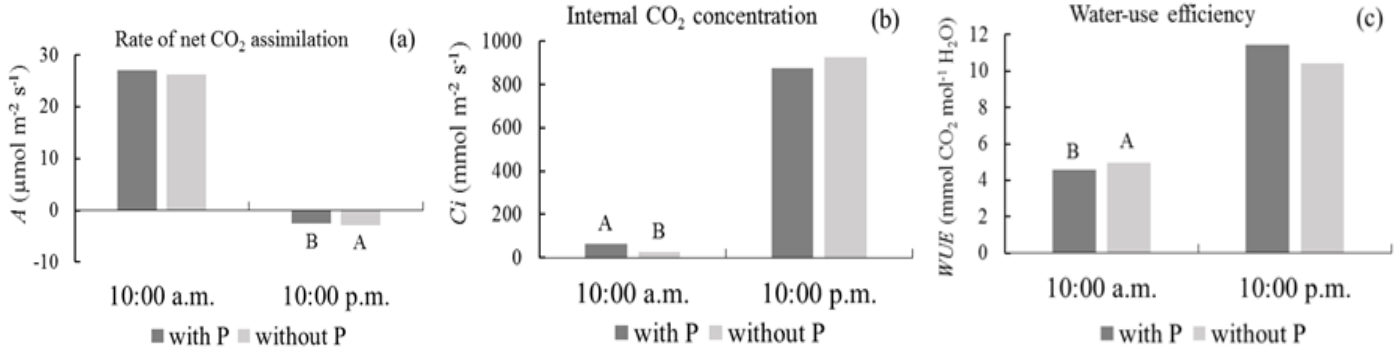

Figure 3. Measures of A = rate of net CO_2 assimilation (a); Ci = internal CO_2 concentration (b) and EUA = water-use efficiency (c), at 10:00 a.m. and 10:00 p.m. from the leaves of plants inoculated via 30F53H hybrid corn seed with plant growth promoting bacteria as a function of phosphate fertilizer.

The evaluation of the *A* measured at 10:00 p.m. showed to be higher for respiration rate when maize plants were not fertilized with phosphorus (Figure 3a). Intercellular CO_2 concentration measured at 10:00 h showed higher value when plants were fertilized with phosphorus (Figure 3b). In turn, the water use efficiency of maize plants showed higher values in the soil devoid of phosphate fertilization (Figure 3c).

One of the main factors that interfere with photosynthesis and leaf transpiration is the difference in the gradient of CO_2 gas and water between the internal and external environment of the leaves (Cowan and Troughton, 1971). The stomata possess the function of intermediating these gas exchanges with opening and stomatal conductance, which is originated by water potential (Farquhar and Raschke, 1978). In this sense, adequate water supply as the crop needs, may have favored similar stomatal behavior of leaf tissue of maize plants.

Conclusion

Phosphorus fertilization improved growth and development of maize plants, regardless of seed inoculation with diazotrophic bacteria. The inoculation with *A. brasilense* and *H. seropedicae* improved the root and shoot growth of maize plants, indicating increase in

the phosphorus solubilization or higher phosphorus use provided by the plant root system.

Seed inoculation with *A. brasilense* associated with phosphorus fertilization enhances the relative chlorophyll content, resulting in higher metabolic structure to the photosynthetic activity of maize plants.

Conflict of Interest

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

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REFERENCES

Alvarez VVH, Fonseca DM (1990). Definition of phosphorus levels to determine the phosphate maximum adsorption capacity and the response curves for greenhouse experiments. R. Bras. Cienc. Solo 14(1):49-55.

Baldani JI, Baldani VLD, Seldin, I, Döbereiner, J (1986). Characterization of Herbaspirillum seropedicae gen. nov., sp. nov., a root-associated nitrogen-fixing bacterium. Int. J. Syst. Bacteriol. Baltimore 36(1):86-93. http://dx.doi.org/10.1099/00207713-36-1-86

Baldani JI, Baldani VLD (2005). History on the biological nitrogen fixation research in graminaceous plants: Special emphasis on the Brazilian experience. An. Acad. Bras. Cienc. 77(3):549-579. http://dx.doi.org/10.1590/S0001-37652005000300014

Baldotto LEB, Silva LGJ, Canellas LP, Olivares F L, Baldotto MA (2012). Initial growth of maize in response to application of rock phosphate, vermicompost and endophytic bacteria. Rev. Ceres 59(2):262-270. http://dx.doi.org/10.1590/S0034-737X2012000200016

Carvalho DDC, Oliveira DF, Pasqual M, Campos VP (2009). Plant growth promoter producing rhizobacteria. Pesquisa Agropecuária Tropical, 39(4):338-341. Disponível em: http://www.revistas.ufg.br/index.php/pat/article/view/3947. Acessado em:10 jul. 2014

Coelho AM, França GE de, Bahia Filho AFC. (2006). Nutrição e adubação do milho. Disponível em: http://www.cnpms.embrapa.br/publicacoes/publica/2006/circular/Circ_78.pdf. Acessado em: 10 jul. 2014.

CONAB – Companhia Nacional de Abastecimento (2014). Indicadores da Agropecuária. Bras. Ano 22(4):90. Disponível em: http://www.conab.gov.br/OlalaCMS/uploads/arquivos/14_04_30_11_38_00_revista__abril_versao_final.pdf. Acessado em: 10 jul. 2014.

Cowan IR, Troughton JH (1971). The relative role of stomata in transpiration and assimilation. Planta, Berlin 97(4):325-336. http://dx.doi.org/10.1007/BF00390212.

Dartora J, Guimarães VF, Marini D, Sander G (2013). Nitrogen fertilization associated to inoculation with Azospirillum brasilense and Herbaspirillum seropedicae in the maize. Rev. Bras. Engenharia Agríc. Ambiental Campina Grande 17(10):1023–1029. http://dx.doi.org/10.1590/S1415-43662013001000001.

Döbereiner J, Baldani VLD, Baldani JI (1995). Como isolar e identificar bactérias diazotróficas de plantas não-leguminosas. Itaguaí: Embrapa - CNPAB P. 60.

Döbereiner J (1953). Azotobacter em solos ácidos. Bol. Inst. Ecol. Exp. Agr. 11:1–36.

Döbereiner J (1966). Azotobacter paspali sp. nov., uma bactéria fixadora de nitrogênio na rizosfera de Paspalum. Pesq Agropec Bras 1:357–365. Disponível em: < http://seer.sct.embrapa.br/index.php/pab/article/view/18041> Acesso em: 11 de jun. 2014.

Döbereiner J, De-Polli H (1980). Diazotrophic rhizocoenoses. Stewart D. P. & Gallon, J. R., ed. Nitrogen fixation. London: Academic Press, 1:301-334.

Döbereiner J, Ruschel AP (1958). Uma nova espécie de Beijerinkia. Rev. Biol. 1:261–272.

EMBRAPA (2013). Empresa Brasileira de Pesquisa Agropecuária. Sistema brasileiro de classificação de solos. 3rd ed. Bras. EMBRAPA P. 353.

Farquhar GD, Raschke K (1978). On the resistance to transpiration of the sites of evaporation within the leaf. Plant Physiology, Lancaster, 60(6):1000-1005. Disponível em: <http://www.ncbi.nlm.nih.gov/pubmed/16660404> Acesso em: 12 de jun. 2014.

Ferreira DF (2011). Sisvar: A computer statistical analysis system. Cienc. Agrotecnol. 15(6):1039-1042. http://dx.doi.org/10.1590/S1413-70542011000600001.

IBGE - Instituto Brasileiro de Geografia e Estatística (2014). Disponível em: http://www.ibge.gov.br/estadosat/temas.php?sigla=pr&tema=lavouratemporaria2011] Acesso em: 10 jul. 2014.

Jain R, Saxena J, Sharma V. (2010). The evaluation of free and encapsulated Aspergillus awamori for phosphate solubilization in fermentation and soil-plant system. Appl. Soil Ecol. 46(1):90-94. http://dx.doi.org/10.1016/j.apsoil.2010.06.008.

Lana M do C, Dartora J, Marini D, Hann JEH (2012). Inoculation with Azospirillum, associated with nitrogen fertilization in maize. Rev. Ceres 59(13):399-405. http://dx.doi.org/10.1590/S0034-737X2012000300016.

Marcos Filho J (2005). Fisiologia de sementes de plantas cultivadas. Piracicaba: Fealq P. 495.

Novais RF, Smyth TJ, Nunes FN (2007). Fósforo. In: Novais R.F., Alvarez V.H., Barros N.F., Fontes R.L.F., Cantarutti R.B., Neves J.C.L. (Eds.) /Fertilidade do solo. Viçosa: Soc. Bras. Ciênc. Solo pp. 471-550.

Rodrigues LFOS, Guimarães VF, Silva MB, Pinto Jr AS, Klein J, Costa ACPR (2014). Características agronômicas do trigo em função de Azospirillum brasilense, ácidos húmicos e nitrogênio em casa de vegetação. Rev. Bras.Engenharia Agríc. Ambiental, Campina Grande 18(1):31-37. http://dx.doi.org/10.1590/S1415-43662014000100005.

Strzelczyk E, Kamper M, LI C. (1994). Cytokinin-like-substances and ethylene production by Azospirillum in media with different carbon sources. Microbiol. Res. 149(1):55-60. http://dx.doi.org/10.1016/S0944-5013(11)80136-9.

Strzelczyk E, Pokojska-Burdziej A (1984). Production of auxins and gibberellin-like substances by mycorrhizal fungi, bacteria and actinomycetes isolated from soil and the mycorrhizosphere of pine (*Pinus silvestris* L.). Plant Soil 81(2):185-194. http://dx.doi.org/10.1007/BF02197150.

Taiz L, Zeiger E (2013). Assimilação de Nutrientes. In: Taiz, L.; Zeiger, E. Fisiologia Vegetal. Porto Alegre: Artmed P. 918.

USDA – United States Department of Agriculture. (2014). Wold Agricultural Supply and Demand Estimates. Disponível em: <www.usda.gov/oce/commodity/wasde/index.htm>. Acesso em: 22 mai. 2014.

Walpola BC, Yoon MH (2013). Phosphate solubilizing bacteria: Assessment of their effect on growth promotion and phosphorous uptake of mung bean (*Vigna radiata* [L.] R. Wilczek). Chilean J. Agric. Res. 73(3):275-281. http://dx.doi.org/10.4067/S0718-58392013000300010.

Murty MG, Ladha JK (1988). Influence of Azospirillum inoculation on the mineral uptake and growth of rice under hydroponic conditions. Plant Soil 108(2):281-285. http://dx.doi.org/10.1007/BF02375660

Souchie EL, Saggin Jr OJ, Silva EMR, Campello EFC, Azcón R, Barea JM (2006). Communities of P-solubilizing bacteria, fungi and arbuscular mycorrhizal fungi in grass pasture and secondary forest of

Paraty, RJ-Brazil. Anais da Academia Brasileira de Ciências, 78(1):183-193. http://dx.doi.org/10.1590/S0001-37652006000100016.

Hameeda B, Harini G, Rupela OP, Wani SP, Reddy G (2008). Growth promotion of maize by phosphate-solubilizing bacteria isolated from composts and macrofauna. Microbiol. Res. 163(2):234-242. http://dx.doi.org/10.1016/j.micres.2006.05.009.

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Full Length Research Paper

Determination of genetic distances in spring wheat by cluster analysis in Mazandaran province (North of Iran)

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Forty-eight genotypes were planted in BIEKOL station field in 2009-2010. This study was carried out in augmented design with three check cultivars in three blocks (a total of 57 genotypes). In order to know uniformity, variance analysis of check genotypes showed that replications were not significant indicating no need for genotypes correction. Mean, standard deviation and coefficient variation for traits were different. The most and the least % CV were related to yield and duration of heading. In cluster analysis, yield components had important roles in cluster distinction. These traits became significant in variance analysis of clusters. In this case, cluster 6 with 8 and cluster 1 with 21 members had the most means in traits.

Key words: Wheat, coefficient of variation, mean, standard deviation, cluster.

INTRODUCTION

Mazandaran Province is a Caspian province in the north of Iran. Located on the southern coast of the Caspian Sea, it is bordered clockwise by the Golestan, Semnan, Tehran, Alborz**,** Qazvin, and Gilan provinces. However, while wheat is grown in over 60,000 ha in this province, its economic value is smaller than that of rice and citrus. Genetic diversity of the wheat landraces must be investigated for use in wheat breeding. More information about the genetic diversity within and relationships among landraces would be invaluable for the conservation and utilization of existing genetic resources. As regards wheat origin, Iran is one of the locations of diversity of common wheat with a long cultivation history. In addition, wheat genetic resources are sent from CIMMYT to Mazandaran Agricultural Research Center as international nurseries.

Basically, calculation of cluster numbers is based on numbers of principal components; however, 4 clusters were selected on the basis of Squared Euclidean Distance cluster because of the more obtained groups to each other than to those in other groups. Thus it can be used to distinguish genetic similarity or distance in wheat genotypes. Wheat has vast genetic diversity in aspects of quantitative and qualitative traits, environmental adaptability and types of tolerances Poelman (1987). Based on Hair et al. (1995) finding, acceptable genetic distance of within cluster should be less than that between clusters. Principal components analysis (PCA)

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Table 1. Pedigree of studied genotypes.

R	***Pedigree (Rep 1)***
1	*ATTILA/3*BCN*2//BAV92*
2	DARYA (SHA4/CHIL)
3	SW89.3243/PRINIA/4/PRINIA/WEAVER//STAR/3/WEAVER
4	BAV92/5/CNDO/R143//ENTE/MEXI75/3/AE.SQ/4/2*OCI
5	PF74354//LD/ALD/4/2*BR12*2/3/JUP//PAR214*6/FB6631/5/SW89-5124*2/FASAN/6/TILH
6	SHA 7//HAHN"S"*2/PRL"S"/3/VEE/NAC
7	NANJING2149/KAUZ/4/JUP/ALD"S"//KIT"S"/3/VEE"S"/5/SHA 7//HAHN"S"*2/PRL"S**"**
8	MILAN CM 75118 // KACM 75118/K1/3/TAJAN(DH**(**
9	BERSEE/3/AZD/VEE"S"//SERI82/ROSH/4/BLOUDAN/3/BB/7C*2//Y50E/KAL*3/CW84
10	PASTOR//NANJING92149/KAUZ/3/PASTOR
11	PASTOR/FINSI
12	N-80-19 (SW89.3064/STAR CMBW91**…**
13	JUP/ZP//COC/3/PVN/4/TNMU/5/TN
14	MORVARID (MILAN/SHA7**(**
15	KAUZ//ALTAR 84/AOS/3/MILAN/KAUZ/4/HUITES
16	ATTILA/3*BCN//BAV92/3/TILHI
17	PASTOR/3/VORONA/CNO 79//KAUZ
18	VORONA/ CNO 79//KAUZ/3/MILAN
19	MILAN/S87230//BABAX

	Pedigree *(Rep 2)*
20	MILAN CM75118/KA CM75118/K 1//TAJAN
21	CAR//KAL/BB/3/NAC/4/VEE/PJN//2*TUI/5/MILAN
22	SHA 7//HAHN"S"*2/PRL"S"/3/ATRAK
23	SIRKKU/FINSI
24	SUNSU/PBW343
25	BL2064//SW89-5124*2/FASAN/3/TILHI
26	DARYA (SHA4/CHIL)
27	OASIS/5*BORL95//SIRKKU/3/CHIBIA
28	BRBT1*2//TUI/CLMS
29	MORVARID (MILAN/SHA7**(**
30	MILAN/ATTILA//ATTILA-4Y
31	NANJING 82149/KAUZ/3/PFAU/SERI//BOW
32	FRET2/TUKURU//FRET2
33	MILAN/S87230//BABAX
34	SABUF/7/ALTAR 84/AE.SQUARROSA (224)//YACO/6/CROC_1**…/**
35	N-80-19 (SW89.3064/STAR CMBW91**(…**
36	SW89.2089/BAKHTAWAR94//SW89.3243
37	CBRD/ARA90
38	BAV92/PRINIA//TAM200/PRL

	Pedigree *(Rep 3)*
39	WBLL1*2/KKTS
40	BABAX//ATTILA/3*BCN/3/PASTOR
41	CROC_1/AE.SQUARROSA (205)//KAUZ/3/ATTILA
42	MORVARID (MILAN/SHA7**(**
43	OASIS/SKAUZ//4*BCN/3/2*PASTOR
44	URES/BOW//OPATA/3/ELVIRA/4/SITE/MO/3/VORONA/BAU//BAU
45	LUCO-M//KAUZ/LUCO-M/3/2*PRINIA
46	BABAX/LR42//BABAX*2/3/VIVITSI
47	CAL/NH//H567.71/3/SERI/4/CAL/NH//H567.71/5/2*KAUZ/6/PASTOR

Table 1. Contd.

48	ALDAN/CIANO67//PASTOR
49	DARYA (SHA4/CHIL**(**
50	CM82A.1294/2*KAUZ//MUNIA/CHTO/3/MILAN
51	N-80-19 (SW89.3064/STAR CMBW91**(…**
52	HP1761//SW89-5124*2/FASAN
53	WAXWING*2/KIRITATI
54	GONDO//SHA5/WEAVER/3/PASTOR
55	OR 1/GONDO//ESDA/LIRA
56	OASIS/SKAUZ//4*BCN/3/2*PASTOR2
57	WBLL1*2/BRAMBLING

Nos. 2, 12, 14, 26, 29, 35, 42, 49, 51 are control cultivars in blocks.

is a method for complement of cluster analysis (Kantety et al., 1995; Johns et al., 1997; Dubreuil and Charcosset, 1998). According to Hailegiorgis and Mesfin (2011), genetic divergence of 49 bread wheat revealed that nine principal components (PC1 to PC9) accounted for nearly 80% of the total variation, thus these genotypes groups are organized into 9 clusters. Fag et al. (1996) used cluster analysis method on 120 wheat genotypes and obtained different groups based on studied traits. van Beuningen and Busch (1997) evaluated 289 spring wheat cultivars from USA, Canada, and Mexico grown in Minnesota during 1990 and 1991 and evaluated in three environments a total of 35 different cultivars.

However, six cultivars could not be grouped into 17 major clusters; major clusters grouped cultivars of common origin, parentage, and/or era of release. The goal of this study is genetic similarity and distance in spring wheat bread by cluster method.

MATERIALS AND METHODS

This trial was carried out in BIE KOLA (Longitude 53, 13° E and Latitude 36°, 43° and 15° meter sea level) station of Mazandaran Agricultural and Natural Resources Center. The experimental materials consisted of fifty-one varieties/lines of spring wheat (Table 1). The experimental materials consisted of 48 varieties/lines of spring wheat with three check cultivars in three blocks, a total of 57 in 3 blocks with 19 genotypes. The most genotypes were international materials from CYMMIT that we investigate every year. The varieties/lines were planted in augmented design. The field area was $6 \times 5 \times 0.2 = 6$ m^2. Studied traits including; Date of heading (DHE, Days), Plant height (PLH, cm), Spike length (SPL, cm), Stem length (SL, cm), Stem diameter (SD, mm), flag leaf width (FLW, cm), flag leaf length (FLL), first- inter-node (FDN), second-inter-node (SDN), third inter-node (TDN), forth inter-node (FODN), spikes per m^2 (SM, No.) Seeds per spike (SS, No.), kernel weight 1,000 (KW, g), yield (yield, gm^{-2}), biomass (BIO, g) and harvest index (HI, %). Used methods were:

1) Calculation of yield means, standard deviation and coefficient variation of traits.
2) Analysis of variance to obtain clusters based on traits.
3) Genotypes clustering based on traits.

To ensure uniformity of blocks, simple variance analysis method was used for yield of checks Milan/Sha4, Sw89.3064/STAR, Sha4/Chil by MSTATC program and in order to estimate relationships between traits, which include yield means, standard deviation and coefficient variation of traits and cluster analysis SPSS program was used (Table 5).

RESULTS AND DISCUSSION

In order to determine traits, divergence was carried out in the calculations as shown in Table 2. **CV%** is a parameter which is not related to unit of measured traits and will be effective in comparing the studied traits. **CV**% of the traits varied from 2% for DHE to 38% for Y (Table 2). Obvious differences of **CV%** values among genotypes declared that genotypes had genetic divergence in some traits. Aghaee et al. (2010) reported that DHE had the lowest **CV%,** while yield and weight of seeds per spike has the most **CV%** value. In this case, results showed that coefficient of variation of two traits (PLH) and (DHE) were little, which can be through favorable rainfall. Variation was observed in yield versus yield components. Cluster analysis was used for grouping of genotypes by UPGMA and Euclidean distance method. Table 3 indicated analysis of variance of 6 clusters based on traits. Distribution pattern of all the genotypes into various clusters showed the presence of considerable genetic divergence among the genotypes for most of the studied .traits. Maximum and minimum mean data were observed within cluster 6 and 1 respectively. Thus, selected clusters had high yield, high yield components and high harvest index. In this way, we selected cluster 6, that is, genotypes with the highest yield and yield components in all clusters. Cluster 1 was the lowest group in yield and yield components (Table 4 and Figure 1). Kumar and Lal (2009) used multiple cluster for selecting of genotypes and on the basis of cluster means, he reported cluster 6 has been identified for selecting parents for incorporating grain yield per plant, tillers per plant and plant height; cluster 5 for spike length, grains per spike and early maturity, and cluster 3 for 1,000 grain weight. Selection of plants was argued on the basis of character associations. Chai (2000) reported that results using Euclidean

Table 2. The estimated mean, standard deviation and coefficient of variation (%CV) of quantitative traits for genotypes.

Variables	Mean	Standard deviation	%CV
DHE	119.0	2.42015	2
PLH	93.1228	6.97718 7	
SL	82.0000	7.09376	9
SPL	9.9298	0.91651	9
SD	4.5754	0.51592	11
FDN	6.9298	2.05165	30
SDN	12.3509	3.70806	30
TDN	19.2105	3.47946	18
FODN	43.2982	6.11487	14
FLL	22.9439	3.08961	13
FLW	1.8053	.21665	12
SM	355.0877	73.64206	21
SS	52.0351	9.83246	19
KW	32.7807	6.50128	20
Y	490.5017	188.02595	38
BIO	1057.8172	195.85921	19
HI	39.6491	7.61223	19

Duration of heading (DHE), plant height (PLH), stem length (SPL), stem-diameter (SD), first-inter node (FDN), second-inter node (SDN), third inter node (TDN), forth inter node (FODN), flag leaf length (FLL), flag leaf width (FLW), spike No. per m^2 (SM), seeds.per spike (SS), kernel weight 1000 (KW), yield (yield), biomass (BIO) and harvest index (HI).

Table 3. Analysis variance of Cluster for studied genotypes on basis of traits.

Traits	Mean square	df
DHE	7.255	5
PLH	48.409	5
SL	50.195	5
SPL	0.531	5
SD	0.318	5
FDN	0.615	5
SDN	12.887	5
TDN	14.394	5
FODN	18.071	5
FLL	11.740	5
FLW	0.101*	5
SM	41163.43**	5
SS	453.038**	5
KW	247.429**	5
Y	337766.8**	5
BIO	371594.9**	5
HI	223.611**	5

Duration of heading (DHE), plant height (PLH), stem length (SPL), stem-diameter (SD), first-inter node (FDN), second-inter node (SDN), third inter node (TDN), forth inter node (FODN), flag leaf length (FLL), flag leaf width (FLW), spike No. per m^2 (SM), seed per spike (SS), kernel weight 1000 (KW), yield (yield), biomass (BIO) and harvest index (HI).

Table 4. Six obtained clusters with number of members.

No. of clusters	No. of members of clusters
1	21
2	12
3	7
4	4
5	5
6	8

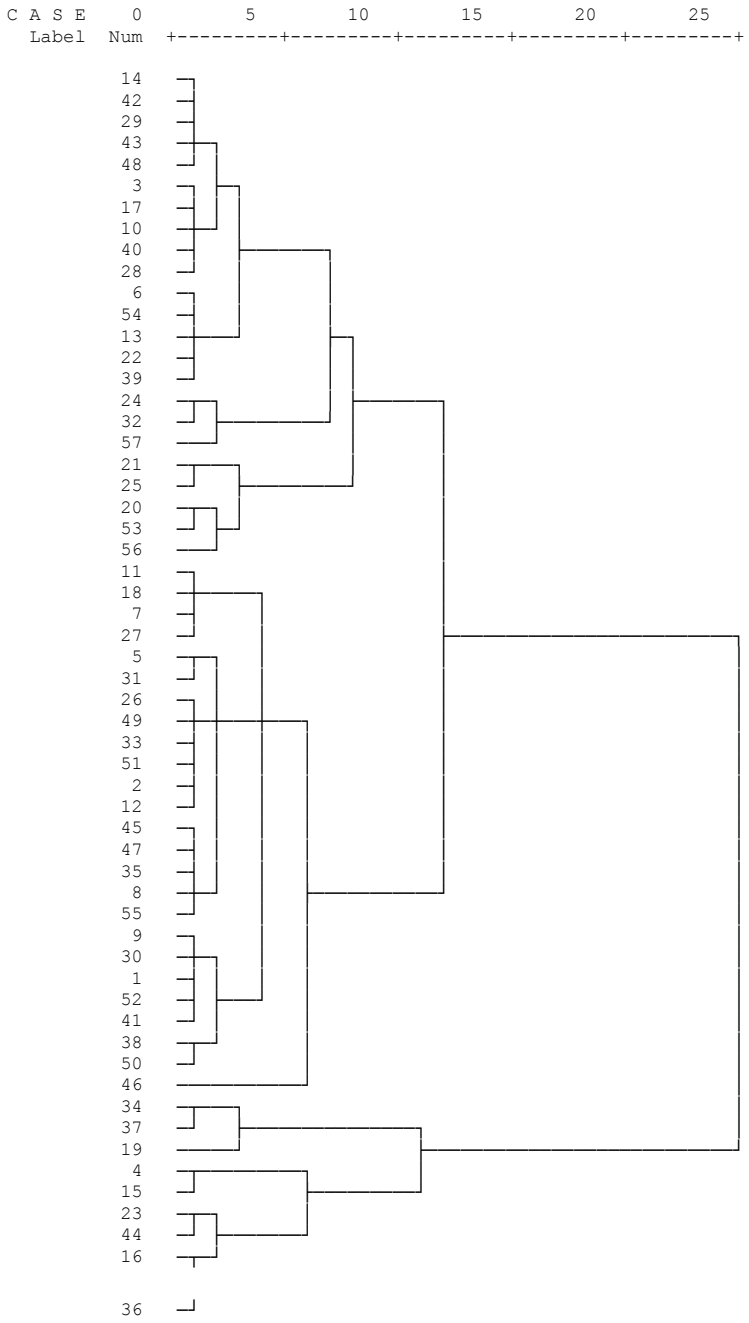

Figure 1. Genotypes grouping in basis of studied.

Table 5. Means of studied traits in six obtained clusters.

Variables	1	2	3	4	5	6
DHE	118.75	118.00	118.25	117.86	118.83	119.95
PLH	96.25	93.6	88.00	91.86	94.75	92.29
SL	85.38	81.4	77.00	80.00	83.50	81.62
SPL	9.54	10.12	9.65	9.93	10.21	9.93
SD	4.30	4.24	4.63	4.76	4.63	4.66
FDN	6.94	6.40	7.50	7.14	6.92	6.88
SDN	12.69	15.10	9.88	12.07	12.17	12.24
TDN	20.06	18.60	16.75	17.21	19.83	19.81
FODN	44.44	41.30	42.88	43.57	45.08	42.31
FLL	24.50	20.80	21.13	23.50	23.21	22.87
FLW	1.84	1.52	1.83	1.87	1.78	1.85
SM	254.63	421.40	500.00	391.86	361.25	334.19
SS	49.13	35.60	57.50	60.00	49.17	55.00
KW	27.21	23.50	40.43	37.63	30.87	35.13
Y	275.7	277.54	922.73	697.57	432.06	505.09
Bio	972.3	695.2	1210.5	1348.4	897.6	1142.3
HI	34.8	28.6	46.8	41.9	40.83	41.38

Duration of heading (DHE), plant height (PLH), stem length (SPL), stem-diameter (SD), first-inter node (FDN), second-inter node (SDN), third inter node (TDN), forth inter node (FODN), flag leaf length (FLL), flag leaf width (FLW), spike No. per m^2 (SM), seeds per spike (SS), kernel weight 1000 (KW), yield (yield), biomass (BIO) and harvest index (HI).

Distance were greater than those using Mahalanobis distance.

Conflict of Interest

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

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REFERENCES

Chai S (2000). Cluster analysis methods appropriate for classification of drought-resistant wheat ecotypes. Ying Yong Sheng Tai Xue Bao, 11(6):833-838. PMid:11767553

Dubreuil P, Charcosset A (1998). Genetic diversity within and among maize populations: A comparison between isozymes and nuclear RFLP loci. Theor. Appl. Genet. 96:577-587. http://dx.doi.org/10.1007/s001220050776

Fag XW, Xiong EH, Zhu W (1996). Cluster analysis of elite wheat germplasm. Jiang Agric. Sci. 4:14-16.

Hailegiorgis T, Mesfin M (2011). Genetic divergence analysis on some bread wheat genotypes grown in Ethiopia. J. Central Eur. Agric. 12:344-352.

Hair JR, Anderson RE, Tatham RL, Black WC (1995). Multivariate data analysis with readings. 4th edition, Prentice-Hall, Englewood Cliffs, NJ. PMCid:PMC2549635

Johns MA, Skrotch PW, Neinhuis J, Hinrichsen P, Bascur G, Munoz-Schick (1997). Gene pool classification of common bean landraces from Chile based on RAPD and morphological data. Crop Sci. 37:605-613. http://dx.doi.org/10.2135/cropsci1997.0011183X003700020049x

Kantety RV, Zeng X, Jeffrey LB, Zehr BE (1995). Assessment of genetic diversity in dent popcorn (*Zea mays* L.) inbred lines using inter-simple sequence repeat (ISSR) amplification. Mol. Breed. 1:365–373. http://dx.doi.org/10.1007/BF01248414

Kumar B, Lal GM (2009). Genetic variability, diversity and association of quantitative traits with grain yield in bread w heat (*Triticum aestivum* L.). Asian J. Agric. Sci. 1(1):4-6

Poelman JM (1987). Breeding Field Crop . Van Nostrand Reinhold. New York. P. 724. http://dx.doi.org/10.1007/978-94-015-7271-2 PMid:3653086

van Beuningen LT, Busch RH (1997). Genetic diversity among North American Spring Wheat Cultivars: III. Cluster Analysis Based on Quantitative Morphological Traits. Alliance of crop, soil. Environ. Sci. Soc. 37:981-988.

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Full Length Research Paper

Response of biofertilizers and homo-brassinolide on growth, yield and oil content of sunflower (*Helianthus annuus* L.)

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Sunflower is an important oil crop in India. The present study aims to find out the response of biofertilizers and homo-brassinolide on growth, yield and oil content of sunflower (*Helianthus annuus* L.). The results showed that inoculation of biofertilizers significantly affected plant height, total chlorophyll content and also significantly increased yield attributes viz., thalamus diameter, weight of thalamus, filled seeds capitulum^{-1} and 1000 seed weight as well as seed yield, biological yield and oil content. The combined inoculation of Phosphate solubilizing bacteria (PSB) + Vesicular-arbuscular mycorrhizal (VAM) + Azotobacter recorded higher seed yield (3189 and 3263 kg ha^{-1}) over Azotobacter (1866 and 2073 kg ha^{-1}), PSB + Azotobacter (2269 and 2421 kg ha^{-1}) and VAM + Azotobacter inoculation (2545 and 2752 kg ha^{-1}) respectively, during the both years. Twice sprayings of homo-brassinolide at budding + flowering stages significantly recorded higher value of plant height, total chlorophyll content and yield parameters as compared to brassinolide spraying at budding stage alone. The maximum seed yield (2760 and 2917 kg ha^{-1}) was obtained from crop receiving both spraying of homo-brassinolide at budding + flowering stages over only one spraying at budding stage (2171 and 2337 kg ha^{-1}) respectively, during 2010-2011 and 2011-2012.

Key words: Azotobacter, brassinolide, phosphate solubilizing bacteria (PSB), Sunflower, vesicular-arbuscular mycorrhizal (VAM).

INTRODUCTION

Sunflower (*Helianthus annuus* L.) is one of the most important oilseed crops containing high quality edible oil. Sunflower kernel has 50% oil content, of which 30% is the essential fatty linoleic acid (Oraki et al., 2011). It is easily cultivated and grown in different conditions and soils (Kaya and Kolsarici, 2011; Lopez-Valdez et al., 2011). Sunflower oil has excellent nutritional properties (Seiler, 2007). Sunflower is one of the fastest growing oilseed crops in India popularly known as "Surajmukhi." Majority of the present day varieties grown all over the world is originated from former USSR. In India, sunflower as an oilseed crop was introduced in 1969. Prior to which it was used mainly as an ornamental plant. Sunflower oil is popular as healthy cooking oil due to its health benefits, while the meal is used in animal feed industry and also being used in the manufacture of soap and

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vanishes (Lawal et al., 2011). Among sunflower products, meal is the most traded in world market.

In view of the environmental and health problems arising from chemical fertilizers usage, many attentions have been drawn to the application of biological fertilizers in agriculture. Use of biofertilizers is one of the important components of integrated nutrient management, as they are cost effective and renewable source of plant nutrients to supplement the chemical fertilizers for sustainable agriculture. Several microorganisms and their association with crop plants are being exploited in the production of biofertilizers. *Azotobacter has* been used as biofertilizer because of their positive effects on growth and productivity of plants via several mechanisms including plant hormones production, nitrogen (N_2) fixation, antagonism against phytopathogenic microorganisms and solubilization of nutrients (Rokhzadi and Toashih, 2011).

Phosphorus (P) is one of the most essential plant nutrients for plant growth after nitrogen and required for higher and sustained productivity of oil from sunflower. However, the availability of this nutrient for plants is limited by different chemical reactions especially in tropical and subtropical soils (Mehrvarz et al., 2008). Phosphorus plays a significant role in several physiological and biochemical plant activities like photosynthesis, transformation of sugar to starch and transporting of the genetic characters because phosphorus is a vital component of the substances that are building blocks of genes and chromosomes. Sharma (2002) reported that one of the advantages of feeding the plants with phosphorus is to produce deeper and more profuse roots. Malakooti (2000) reported that phosphorus had an important role in early ripening, decreasing grain moisture, improving crop quality. Arpana et al. (2002) reported that a large proportion of phosphorus in chemical fertilizer becomes unavailable to the crop plants after its application in the soil due to its rapid fixation in soil. Its influence on seed yield, oil yield and oil quality has been well established by Loubser and Human (1993), Bahl and Toor (1999), Chandrashekara et al. (1995) and Zubillaga et al. (2002); also, application of phosphorus has become an essential part of sunflower fertilizer program.

In general, phosphorus is added to soil as inorganic phosphates, because the free inorganic P in soil solution plays a central role in P-cycling and plant nutrition (Peix et al., 2001). However, a large portion of soluble inorganic phosphate applied to soil as chemical fertilizer is immobilized rapidly after application due to phosphate fixation by aluminum, calcium, iron, magnesium and soil colloids (Rodriguez and Fraga, 1999) and becomes unavailable to plants (Singh and Kapoor, 1994). Therefore, P is often a limiting nutrient in agricultural soils. Micro-organisms are also involved in a range of process that affect the transformation of soil P and thus an integral part of the soil P cycle (Chen et al., 2006). In particular, P-solubilizing micro-organisms (bacteria or fungi) are able to solubilize unavailable soil P and increase the yield of crops (Adesemoye and Kloepper, 2009). Plant growth-promoting rhizobacteria (PGPR) and rhizosphere bacteria are free-living soil organisms that can benefit plant growth by different mechanisms (Glick, 1995). P-solubilization ability of micro-organisms is considered to be one of the most important traits associated with plant P nutrition (Chen et al., 2006). Several bacterial species, in association with plant rhizosphere are capable of increasing availability of Phosphorus to plants either by mineralization of organic phosphate or by solubilization of inorganic phosphate by production of acids (Rodriguez and Fraga, 1999).

Vesicular-arbuscular mycorrhizal (VAM) fungi are normally known to benefit plant health, with the net benefit to plant increasing as stress increases due to lack of nutrient and soil moisture (Bethlenfalvay and Svejcar, 1991; Sieverding, 1991). VAM fungi contribute greatly to phosphorus uptake (George et al., 1992; Smith et al., 1992) and to nitrogen uptake directly (Azcon-Aguilar et al., 1993). Regardless of the actual mechanism involved, VAM fungi can increase the efficiency of phosphorus and nitrogen removal from the soil solution over that of roots alone, which has obvious implications for reducing fertilizer inputs and leaching. VAM fungi have also been shown to be important in the uptake of other ions, including K, S, Mg, Fe, Z, Cu (Cooper, 1984; Sieverding, 1991). Brassinosteroids (BRs) are naturally occurring steroidal plant hormones and their various forms, widely distributed in the plant kingdom, have a distinct role in stem elongation, pollen tube growth, leaf bending, ethylene biosynthesis, and xylem differentiation (Khripach et al., 2002). The standing plants supplemented with BRs improved the productivity potential of economically important crops by activating cell elongation, vascular differentiation, and/or proton pump (Rao et al., 2002). Plants supplemented with BRs exhibited an increase in the activities of carbonic anhydrase and nitrate reductase (Hayat et al., 2001; Hayat et al., 2001a), phosphoenolpyruvate carboxylase, ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco), and the contents of soluble proteins (Braun and Wild, 1984; Braun and Wild, 1984a; Yang et al., 1992). BRs treated plants also exhibit higher resistance to stress and produce more seeds in crop plants (Rao et al., 2002). Brassinosteroids (BRs) are regarded essential for the normal growth and development of plants (Li and Chory, 1999). Hence, an experiment was conducted to study the response of biofertilizers and homo-brassinolide phytohormone on growth, yield and oil content of sunflower (*H. annuus*. L).

MATERIALS AND METHODS

A field experiment was conducted during the winter season (October to March) of 2010-2011 and 2011-2012 at farmers field

adjacent to the farm of the Institute of Agriculture (Palli Siksha Bhavana), Visva-Bharati, Sriniketan, West Bengal. The place is situated at 23°39' N latitude, 87°42' E longitude and an elevation of 58.9 m above mean sea level. The experiment was established in sandy loam soil with pH 5.7, low in available nitrogen (130 kg ha^{-1}), phosphate (12.50 kg ha^{-1}) and medium in potassium (163.5 kg ha^{-1}). Seed bed preparation included ploughing, disk harrowing and cultivation. Sowing was performed manually.

The experiment was laid out in factorial randomized block design with four types of biofertilizers inoculation (Azotobacter, Phosphate solubilizing bacteria (PSB) + Azotobacter, Vesicular arbuscular mycorrhizae (VAM) + Azotobacter and Phosphate solubilizing bacteria (PSB) + Vesicular arbuscular mycorrhizae (VAM)) + Azotobacter and two spraying of homo-brassinolide (HBR) @ 0.5 ml L^{-1} of water at 50% budding stage and 50% budding + 50% flowering stages. In all, eight treatments were replicated three times. Each plot consisted of ten rows, and each row was 5 m long. The first and last rows in each plot were considered as marginal effects. The seed was inoculated with Azotobacter and PSB by slurry method whereas the soil was inoculated with VAM inoculums (Manufactured by Symbiotic Sciences, New Delhi). The VAM inoculums were placed at the seeding depth of the soil and then pre-inoculated seeds were sown according to the treatment. Homo-brassinolide (DOUBLE) was used according to the treatment. Plots were fertilized with the same amount of fertilizer as 80 kg ha^{-1}N, 100 kg ha^{-1} P_2O_5 and100 kg ha^{-1} K_2O but ½th nitrogen fertilizer was added before sowing as basal dose and rest amount was added before bud formation.

In the study, "PAC 36" commercial hybrid of oilseed sunflower, which had early to medium maturation, high yield potential, responsive to higher inputs, more tolerant to diseases and pests, higher drought tolerance, more self fertile, superior in their seed filling ability and higher adaptation ability, was used as plant material. Seeds were sown in rows 45 cm apart and plant to plant distance 20 cm, using 5 kg seed ha^{-1}.

The sunflower plants were harvested by hand, from middle seven rows excluding side rows and 1 m from each end of plots at the stage of physiological maturation when the back of the head had turned from green to yellow and the bracts were turning brown (Anonymous, 2005) or fruit dry weight (FDW) has reached its maximum value with a water content about 38% (Rondanini et al., 2007). Plant height and head diameter were determined at physiological maturity, by harvesting 10 plants of sunflower per experimental unit. Aerial dry matter production was determined at 45, 60, 75 and 90 DAS, by cutting 5 plants at ground level from each plot kept in a hot air oven at 65°C for 48 h till constant weight was obtained. The dry weight of plants were recorded and used for determination of aerial dry matter production. Heads were separated from vegetative parts. Head diameter was estimated for distance in cm across the apical head at its widest point. The head samples for yield were also dried at 60°C for 48 h in hot air oven to get constant weight and threshed mechanically. Seed yield was adjusted to a 10% moisture basis. Filled grain and empty hulls were separated by hand. Hereafter, grain number per head refers to filled grains only. Harvest index was computed by dividing the seed yield from the total biological yield and was expressed as percentage. The harvest index (HI) was calculated by the formula given by Donald (1962).

$$HI = \frac{\text{Economic yield}}{\text{Biological yield}} \times 100\ (\%)$$

Seed oil contents were determined using the Soxhlet and Kjeldahl method in seed kernel (dehulled). Fully open fresh new leaves were clipped from plants of each plot at morning hours and immediately brought to the laboratory in a plastic bag for estimation chlorophyll content at 30, 45, 60 and 75 DAS. Total chlorophyll content was measured adopting the method of Hiscox and Israelstam (1979), using Dimethyl sulfoxide (DMSO). The chlorophyll content was determined using the formula given by Arnon (1949) and expressed as mg g^{-1} of fresh leaf. Arnon's formula estimate total chlorophyll as follows.

Total Chlorophyll = $[20.2(D_{645}) + 8.02\ (D_{663})] \times V/[1000 \times W]$,

Where, D = Absorbance, V = Final volume of DMSO (ml); W = Weight of fresh leaf (g)

Statistical Analysis

Data collected were subjected to statistical analysis of variance according to Gomez and Gomez (1984) using MSTAT computer program.

RESULTS AND DISCUSSION

Plant height

Effect of biofertilizers and homo-brassinolide on plant height at harvest stage of sunflower is presented in Table 1. The tallest plant height (118.6 and 121.4 cm) was recorded with inoculation of PSB + VAM + Azotobacter over other biofertilizers treatment during 2010-11 and 2011-12 seasons, respectively. There was no significant difference between the treatments of PSB + Azotobacter and VAM + Azotobacter in respect of plant height during both years. The dwarf plant height (72.9 and 78.9 cm) was obtained from crop receiving Azotobacter alone during both years and it was significantly lowest than other biofertilizers treatments. The tallest height might be due to the strong synergistic effect of PSB + VAM + Azotobacter. Inoculations of PSB which are known to produce growth hormones like IAA and GA (Sattar and Gaur, 1987) are likely to favor increased plant height. The results are in conformity with those of Mukherjee and Rai (2000). Twice sprayings of homo brassinolide at budding + flowering stages significantly improved plant height as compared to one spraying of homo brassinolide at budding stage. Increased plant height might be due to positive effect of homo-brassinolide on meristamatic tissues of plant as well as in increasing number and size of cell (Prakash et al., 2008). Similar result was reported by Mitchell and Gregory (1972).

Aerial biomass production

Data on crop aerial biomass production at different growth stages (45, 60, 75 and 90 DAS) are presented in Table 1. Scrutiny of records during all the observations revealed that the maximum aerial biomass production (9.39 to 53.45 g $plant^{-1}$) of crop was obtained using PSB + VAM + Azotobacter as compared to other treatment or treatment combinations irrespective of the growth stages during both years. The minimum aerial biomass production (4.25 to 23.88 g $plant^{-1}$) of crop was obtained from the crop receiving Azotobacter and it was

Table 1. Plant height and aerial biomass (g plant^{-1}) of Sunflower at different growth stages as influenced by biofertilizers and homo-brassinolide.

Treatments	Plant height at harvest		Aerial biomass (g plant^{-1})							
			45 DAS		60 DAS		75 DAS		90 DAS	
	2010-11	2011-12	2010-11	2011-12	2010-11	2011-12	2010-11	2011-12	2010-11	2011-12
Bio-fertilizers										
Azotobacter	72.9	78.9	3.87	4.25	11.60	12.40	17.80	18.60	21.85	23.88
PSB + Azotobacter	95.7	99.2	7.00	7.21	15.62	16.14	25.07	27.27	35.24	39.79
VAM + Azotobacter	100.5	104.2	7.12	7.55	16.89	17.47	26.12	27.74	36.35	40.51
PSB+VAM+Azotobacter	118.6	121.4	9.12	9.39	21.80	22.38	33.98	36.17	48.53	53.45
S.Em±	2.22	1.99	0.17	0.15	0.47	0.48	0.60	0.56	0.78	0.83
C.D. (P=0.05)	6.75	6.04	0.54	0.46	1.44	1.46	1.81	1.71	2.38	2.52
Homo-brassinolide										
Budding stage	90.4	95.2	6.76	7.04	16.33	17.09	23.92	24.93	32.58	35.49
budding + flowering stage	103.4	106.7	6.79	7.15	16.62	17.10	27.56	29.95	38.39	43.32
S.Em ±	1.57	1.40	0.12	0.11	0.33	0.47	0.42	0.40	0.55	0.58
C.D. (P=0.05)	4.77	4.27	NS	NS	NS	NS	1.28	1.21	1.68	1.78

significantly lower than other biofertilizers treatment. The high response of plant to the PSB + VAM + Azotobacter inoculation might be due to solubilization and mobilization of available P by the native soil microflora, or attributed due to increased PSB and VAM activity in the rhizosphere and consequently enhanced P solubilization and mobilization. Therefore, increased aerial biomass production of crop by PSB + VAM + Azotobacter might be due to better development of root systems resulting in tapping larger volume of bound soil water and nutrients especially phosphorus. This result is in conformity with the findings of Mukherjee and Rai (2000). Homo-brassinolide level exerted significant positive effect on aerial biomass production. The maximum aerial biomass production was recorded in crop receiving spraying of homo-brassinolide at budding + flowering stages at 75 and 90 DAS over one spraying of homo-brassinolide at budding stage. There was no effect of homo-brassinolide because it was applied after 60 DAS. Increased aerial biomass production of crop with homo-brassinolide might be due to higher plant growth as well as more positive effect on meristamatic tissues of plant and in increasing number and size of cell (Prakash et al., 2008). Homobrassinolide (HBR) is one of the bioactive brassinosteriods considered as plant hormone with pleiotropic effects as they influence on developmental processes such as growth (Sasse, 1999).

Total chlorophyll content

Data on total chlorophyll content in leaf as affected by application of biofertilizers and homo-brassinolide are presented in Table 2. Data also indicated that total chlorophyll content increased with advancement of the crop age up to 60 DAS and thereafter marginally decreased at 75 DAS. The decrease in the amount of chlorophyll contents suggested that the leaves were at their senescence stage. During the process of senescence, the composition of cell components of the leaves are progressively degraded (Erickson, 1968; Sinclair et al., 1971; Craig and Shih, 1998). Inoculation of crop with PSB + VAM + Azotobacter recorded significantly higher total chlorophyll content at 75 DAS. This might be due to higher content of nitrogen and magnesium which are the core component of chlorophyll (Ruiz-Lozano and Azicon, 1995). This finding is generally in agreement with the previous finding of Demir et al. (2011). Spraying of homo-brassinolide significantly influenced total chlorophyll content at 75 DAS. Twice spraying of homo-brassinolide at budding + flowering stage recorded significantly higher total chlorophyll content (1.89 and 1.95 mg g^{-1} of fresh leaf) as compared to one spray of budding stage (1.53 and 1.62 mg g^{-1} of fresh leaf). This might be due to better vigorousness of root system with consequent supply of nitrogen from soil. It has been evidenced that there is a positive correlation between nitrogen and total chlorophyll content. These results are in agreement with the findings of Ramraj et al. (1997), Vardhini and Rao (1998) and Nakashita et al. (2003).

100 seed weight

The highest 100 seed weight was obtained with inoculation of PSB + VAM+ Azotobacter (Table 3) over Azotobacter, PSB + Azotobacter and VAM + Azotobacter inoculation. The lowest test weight of 100 seed weight recorded was obtained with Azotobacter inoculation

Table 2. Total chlorophyll content (mg g^{-1} of fresh leaf) of Sunflower at different growth stages as influenced by biofertilizers and homo-brassinolide.

Treatments	30 DAS		45 DAS		60 DAS		75 DAS	
	2010-11	2011-12	2010-11	2011-12	2010-11	2011-12	2010-11	2011-12
Bio-fertilizers								
Azotobacter	1.05	1.07	1.65	1.75	1.94	2.00	1.55	1.58
PSB + Azotobacter	1.14	1.16	1.73	1.79	1.99	2.07	1.60	1.68
VAM + Azotobacter	1.21	1.16	1.77	1.86	2.05	2.34	1.78	1.84
PSB+VAM+Azotobacter	1.30	1.36	2.35	2.16	2.23	2.37	1.93	2.06
S.Em (±)	0.03	0.02	0.04	0.08	0.04	0.03	0.04	0.04
C.D. (P=0.05)	0.09	0.06	0.12	0.26	0.13	0.11	0.12	0.12
Homo-brassinolide								
Budding stage	1.17	1.19	1.87	1.81	2.02	2.17	1.53	1.62
budding + flowering stage	1.18	1.18	1.88	1.96	2.09	2.22	1.89	1.95
S.Em±	0.02	0.01	0.03	0.06	0.03	0.03	0.02	0.03
C.D. (P=0.05)	NS	NS	NS	NS	NS	NS	0.07	0.08

Table 3. Yield components, seed yield, harvest index and oil % of Sunflower at different growth stages as influenced by biofertilizers and homo-brassinolide.

Treatment	100 seed weight (g)		Diameter of thalamus (cm)		Seed yield (kg ha^{-1})			Stalk yield (kg ha^{-1})		Harvest index (%)		Oil %	
	2010-11	2011-12	2010-11	2011-12	2010-11	2011-12	Pooled	2010-11	2011-12	2010-11	2011-12	2010-11	2011-12
Bio-fertilizers													
Azotobacter	4.70	4.72	11.20	11.67	1866	2073	1969	5929	6157	25.54	26.79	30.98	32.14
PSB + Azotobacter	4.87	4.94	12.70	13.48	2264	24.21	2342	6573	6918	27.09	27.32	32.85	33.66
VAM + Azotobacter	4.97	5.06	13.45	14.17	2545	27.52	2648	6935	7200	28.21	29.02	34.17	34.99
PSB+VAM+Azotobacter	5.33	5.44	15.25	16.00	3189	32.63	3225	7582	7544	30.90	31.52	36.52	37.84
S.Em±	0.05	0.06	0.34	0.35	65	59	44	99	107	0.54	0.33	0.44	0.29
C.D.(P=0.05)	0.16	0.18	1.03	1.08	197	181	127	300	324	1.64	1.00	1.35	0.90
Homo-brassinolide													
Budding stage	4.76	4.86	11.93	12.85	2171	2337	22.54	6434	6634	26.62	27.47	32.74	33.44
budding + flowering stage	5.16	5.21	14.36	14.80	2760	2917	28.38	7074	7275	29.24	29.85	34.51	35.87
S.Em±	0.04	0.04	0.24	0.25	45	42	31	70	75	0.38	0.23	0.31	0.21
C.D.(P=0.05)	0.11	0.12	0.72	0.76	139	128	89	212	229	1.16	0.71	0.95	0.64

alone. These results are in agreement with the previous findings of Barea et al. (1975). Twice spraying of homo-brassinolide at budding + flowering stage recorded a significant highest 100 seed weight as compared to one spraying of homo-brassinolide at budding stage. The results are in conformity with those of Mitchell and Gregory (1972).

Diameter of thalamus

Diameter of thalamus recorded was significantly influenced by the combined inoculation of PSB + VAM + Azotobacter (Table 3) as compared to Azotobacter, PSB + Azotobacter and VAM + Azotobacter inoculation. The high response of plant to the PSB + VAM+ Azotobacter inoculation might be due to mobilization of available P by the native soil microflora, or attributed to increased phosphate solubilizing bacteria activity in the rhizosphere following PSB + VAM + Azotobacter application and consequently by enhanced P solubilization. For these reasons, it enhanced P uptake by the crops and an increased thalamus diameter ultimately leading to higher seed yields. Similar result was reported by Barea et al.

(1975). Spraying of homo-brassinolide at budding + flowering stage was significantly highest in diameter of thalamus as compared to one spray at budding stage. The results are in conformity with those of Mitchell and Gregory (1972).

Seed yield

Inoculation of PSB + VAM + Azotobacter showed significant effect on seed yield (Table 3) as compared to Azotobacter, PSB + Azotobacter and VAM + Azotobacter inoculation. The result of pooled analysis showed that combined inoculation of PSB + VAM resulted in 63.78, 37.70 and 21.79% higher seed yield over Azotobacter, PSB + Azotobacter and VAM + Azotobacter inoculation, respectively. This increase in yield parameters by PSB + VAM + Azotobacter inoculation might be due to increase in phosphorus and nitrogen availability, roots, relative water content, root biomass, nodule number and dry weight that could be ascribed to a better translocation of photosynthates towards yield attributes and yield. Photosynthesis generates the high energy sugars. Increased availability of phosphorus provides the mechanism for energy storage in the form of ATP and the transfer of that energy source to fuel vital plant functions such as N fixation. Rose (1957) recorded similar findings. The phosphate solubilizing bacteria is known to produce vitamins (Baya et al., 1981) and IAA- and GA-like growth substance (Satter and Gaur, 1987). The result is in conformity with those of Jones and Sreenivas (1993). Spraying of homo-brassinolide at budding + flowering stage was significant as compared to one spraying at budding stage. The result of pooled analysis showed that two spraying of homo-brassinolide increased 25.90% higher seed yield as compared to one spraying. The increase in yield might be due to the higher photochemical efficiency in terms of hill reaction, and CO_2 intake in twice spraying of homo-brassinolide may be attributed to increased accumulation of photosynthetic pigments particularly chlorophyll and higher soluble protein content. The results are in conformity with those of Nayak and Murthy (1980) and Chowdhary et al. (1994).

Stalk yield

Inoculation of PSB + VAM + Azotobacter recorded significantly higher stalk yield as compared to Azotobacter, PSB + Azotobacter and VAM + Azotobacter inoculation (Table 3). This increase in stalk yield by PSB + VAM + Azotobacter inoculation might be due to better growth of the plants. Shinde (1990) and Yadav and Shrivastava (1997) recorded similar findings. Twice spraying of homo-brassinolide at budding + flowering stage produced significantly higher stalk yield over one spraying of homo-brassinolide at budding stage. The result is in conformity with those of Meudt et al. (1983).

Harvest index

The maximum harvest index was recorded with the combined inoculation of PSB + VAM + Azotobacter (Table 3) followed by Azotobacter + VAM, PSB + VAM and Azotobacter. The minimum harvest index was obtained from the crop receiving only Azotobacter, PSB + Azotobacter and VAM + Azotobacter inoculation. The higher harvest index might be due to better yield with PSB + VAM + Azotobacter. Similar type of result was previously reported by Barea et al. (1975). Spraying of homo-brassinolide at budding + flowering stage showed significant highest harvest index as compared to one spray at budding stage. The results are in conformity with those of Mitchell and Gregory (1972).

Oil content (%)

Combined inoculation of PSB + VAM + Azotobacter recorded significantly higher oil content over Azotobacter + VAM, PSB + VAM and Azotobacter. Our results were similar to the findings of Patra et al. (2013). The spraying of homo-brassinolide at budding + flowering stage recorded maximum oil content whereas Azotobacter recorded minimum oil content in seed The increase in oil content might be due to spraying of homo-brassinolide which was in consonance with the findings of Mai et al. (1989) and Prakash et al. (2008) in rice and sesame respectively. The increase in oil content might be due to increase in morphological, physiological and biochemical parameters (Prakash et al., 2008).

Correlation studies

Looking at the correlation coefficients in Table 4, a significant positive correlation was noted between seed yield and test weight during both years of experiment. There was also a positive relationship between seed yield and chlorophyll content. Sharafai et al. (2006) reported positive and significant relation between grain yield and 100-grain weight in maize. Thus, correlation studies showed that seed yield was positively and significantly correlated with test weight and total chlorophyll content at different stages of crop growth during both years of experiment.

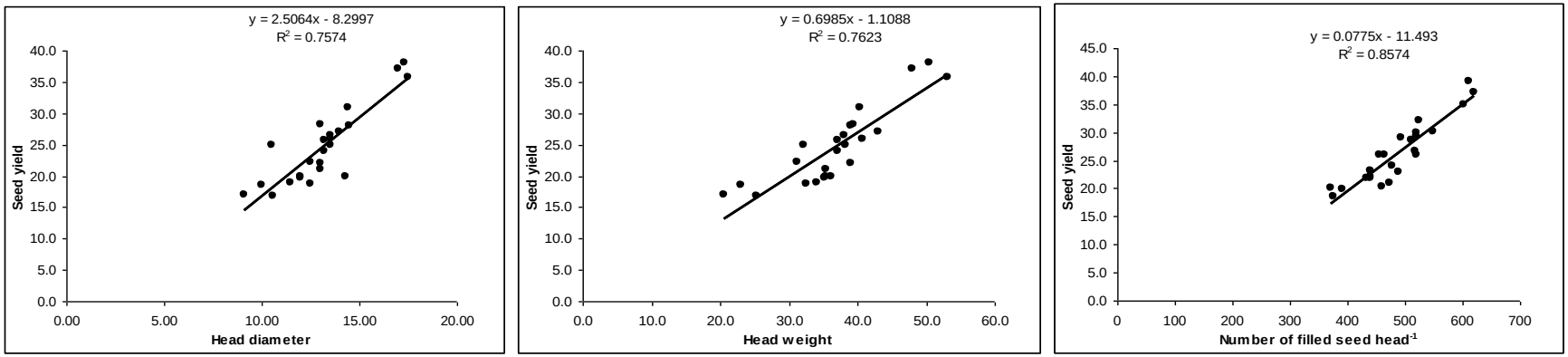
Relationship between yield parameter and seed yield

Head diameter, head weight and number of filled seed $head^{-1}$ showed significant positive association with seed yield during both years (Figures 1 and 2). The increase in

Table 4. Simple correlation coefficient for seed yield and test weight and total chlorophyll content in sunflower during 2010 to 2012.

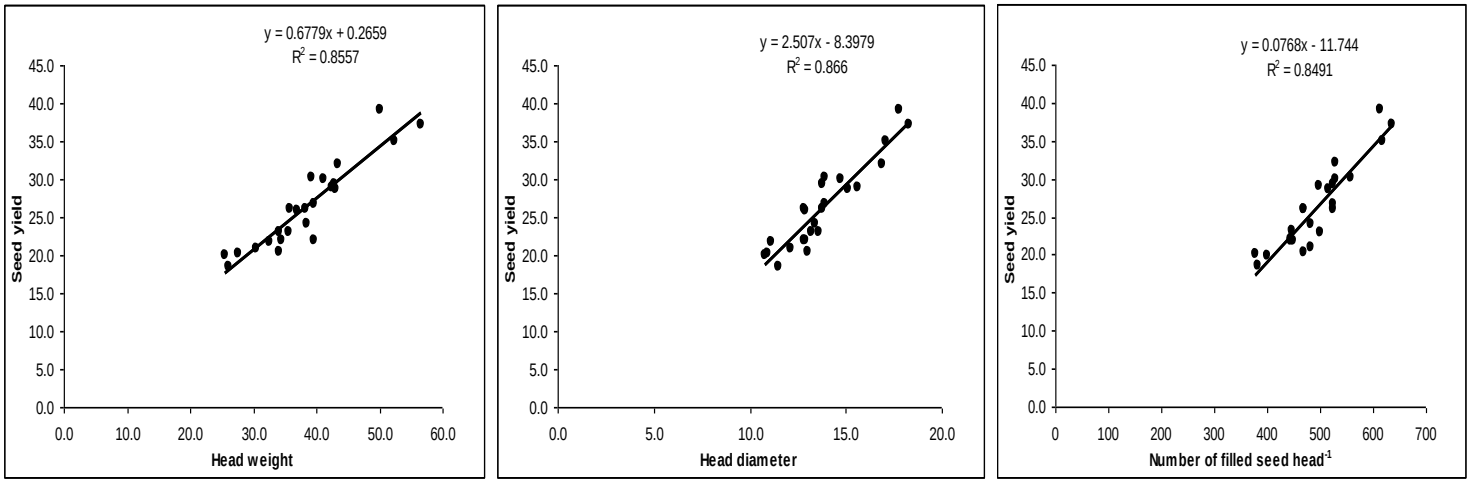
Characters	Years	Seed yield (Y)	Test weight (X_1)	Total chlorophyll content at 30 DAS (X_2)	Total chlorophyll content at 45 DAS (X_3)	Total chlorophyll content at 60 DAS (X_4)	Total chlorophyll content at 75 DAS (X_5)
Y	2010-11	1.000	0.882**	0.757**	0.709**	0.661**	0.855**
	2011-12	1.000	0.868**	0.655**	0.726**	0.700**	0.910**
(X_1)	2010-11		1.000	0.511	0.578	0.693**	0.848**
	2011-12		1.000	0.756**	0.675**	0.669**	0.881**
(X_2)	2010-11			1.000	0.740**	0.397	0.476
	2011-12			1.000	0.711**	0.581	0.636**
(X_3)	2010-11				1.000	0.499	0.510
	2011-12				1.000	0.404	0.734**
(X_4)	2010-11					1.000	0.556
	2011-12					1.000	0.695**
(X_5)	2010-11						1.000
	2011-12						1.000

**Significant at P=0.01 levels.



(a) (2010-11) (b) (2010-11) (c) (2010-11)

Figure 1. Relationship between (a) head diameter (cm), (b) head weight (g) and (c) number of filled seed head^{-1} with seed yield (q ha^{-1}).



(d) (2011-12) (e) (2011-12) (f) (2011-12)

Figure 2. Relationship between (d) head diameter (cm), (e) head weight (g) and (f) number of filled seed head^{-1} with seed yield (q ha^{-1}).

seed yield with increasing head diameter, head weight and number of filled seed head^{-1} was linear in fashion. Head diameter accounted for 75% variability in seed yield during 2010-11 and 86% variability in seed yield during 2011-12. Similarly, head weight accounted for 76% variability in seed yield during 2010-11 and 85% variability in seed yield during 2011-12. On the other hand, number of filled seed head^{-1} accounted for 85% variability in seed yield during 2010-11 and 84% variability in seed yield during 2011-12. Hence, increasing head diameter, head weight and number of filled seed head^{-1} has direct effect on increasing the seed yield of sunflower.

Conclusion

The maximum plant height, aerial biomass production, chlorophyll content, 100 seed weight, diameter of thalamus, seed yield, stalk yield, harvest index and oil content were obtained with inoculation of PSB + VAM + Azotobacter. On the other hand, twice spraying of homo-brassinolide at 50% budding initiation + 50% flowering stages also recorded maximum aerial biomass production, chlorophyll content, 100 seed weight, diameter of thalamus, seed yield, stalk yield, harvest index and oil. The result indicated the importance of adopting suitable combination of biofertilizers and spraying of homo-brassinolide in this experiment lateritic soil. Thus, the appropriate combination of biofertilizers and homo-brassinolide can increase seed yield and improve oil content in sunflower.

Conflict of Interest

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

REFERENCES

Adesemoye AO, Kloepper JW (2009). Plant-microbes interactions in enhanced fertilizer-use efficiency. Appl. Microbiol. Biotechnol. 85:1-12. http://dx.doi.org/10.1007/s00253-009-2196-0

Anonymous (2005). Sunflower Magazine: Harvest Fundamentals. https://www.sunflowernsa.com/Magazine/details.asp?ID=388&Cat=12. Accessed on 16th August, 2014.

Arnon DI (1949). Copper enzyme in isolated chloroplasts polyphenol oxidase in Beta vulgaris. Plant Physiol. 24:1-15. http://dx.doi.org/10.1104/pp.24.1.1

Arpana N, Kumar SD, Prasad TN (2002). Effect of seed inoculation, fertility and irrigation on uptake of major nutrients and soil fertility status after harvest of late sown lentil. J. Appl. Biol. 12:23–26.

Azcon-Aguilar C, Alba C, Montilla M, Barea JM (1993). Isotopic (^{15}N) evidence of the use of less available N forms by VA mycorrhizas. Symbiosis 15:39-48.

Bahl GS, Toor GS (1999). Efficiency of P utilization by sunflower grown on residual P fertility. Bioresour. Technol. 67:97-100. http://dx.doi.org/10.1016/S0960-8524(99)00071-1

Barea JM, Azcon R, Hayman DS (1975). Possible synergistic interaction between Endogene and phosphate solubilizing bacteria in low phosphorus soils. In Endomycorrhizas Eds. F.E. Sanders, B. Mosse and P.B. Tinker. pp. 409-418. Academic press. London. New York.

Baya AM, Boethling RS, Ramos CA (1981). Vitamin production in relation to phosphate solubilization by soil bacteria. Soil Biol. Biochem. 13:527-531. http://dx.doi.org/10.1016/0038-0717(81)90044-4

Bethlenfalvay GJ, Svejcar TE (1991). Mycorrhizae in plant productivity and soil conservation. In: Proceedings of the IV th International Rangelend Congress, Montpellier, France.

Braun P, Wild A (1984). The influence of brassinosteroid on growth and parameters of photosynthesis of wheat and mustard plants. J. Plant Physiol. 116:189-196. http://dx.doi.org/10.1016/S0176-1617(84)80088-7

Braun P, Wild A (1984a). The influence of brassinosteroid on growth promoting steroidal lactone, on development and CO_2-fixation capacity of intact wheat and mustard seedlings In: Sybesma C (ed) Advances in photosynthesis research Vol III, Martinus Nijhoff/ Dr. W Junk Publishers. Hague- Boston-Lancaster pp. 461-464.

Chandrashekara CP, Patil VC, Sreenivasa MN (1995). VA-mycorrhiza mediated P effect on growth and yield of sunflower (*Helianthus annuus* L.) at different P levels. Plant Soil 176:325-328. http://dx.doi.org/10.1007/BF00011797

Chen YP, Rekha PD, Arun AB, Shen FT, Lai WA, Young CC (2006). Phosphate solubilizing bacteria from subtropical soil and their tricalcium phosphate solubilizing abilities. Appl. Soil Ecol. 34:33-41. http://dx.doi.org/10.1016/j.apsoil.2005.12.002

Chowdhary PK, Thangaraj M, Jayapragasam M (1994). Biochemical changes in low-irradiance tolerant and susceptible rice cultivars. Biol. Plantarum 36(2):237-242. http://dx.doi.org/10.1007/BF02921092

Cooper KM (1984). Physiology of VA mycorrhizal associations. In:VA Mycorrhiza, Powell, C.L. and Bagyaraj, D.J., Eds. CRC Press Boca Raton, FL. pp. 155-188.

Craig JC, Shih SF (1998). The spectral response of stress conditions in citrus trees: development of methodology. Soil and Crop Science Society of Florida. 57:16-20.

Demir K, Başak H, Okay FY, Kasım R (2011). The effect of endo-mycorrhiza (VAM) treatment on growth of tomato seedling grown under saline conditions. Afr. J. Agric. Res. 6(14):3326-3332.

Donald CM (1962). In search of high yields. J. Aust. Inst. Agric. Sci. 28:171-178.

Erickson LC (1968). The general physiology of citrus. The Citrus Industry, Anatomy, Physiology, Genetics, and Reproduction, (Eds.): W. Reuhter, L.D. Batchelor and H.J. Webber. 2:86-122.

George E, Haussler K, Vetterlein D, Gorgus E, Marschner H (1992). Waternand nutrient translocation by hyphae of *Glomus mosseae*. Can. J. Bot. 70:2130-2137. http://dx.doi.org/10.1139/b92-265

Glick BR (1995). The enhancement of plant growth by free-living bacteria. Can. J. Microbiol. 41:109-117. http://dx.doi.org/10.1139/m95-015

Gomez KA, Gomez AA (1984). Statistical procedure for Agricultural Research 2nd Edition, John Wiley and Sons.

Hayat S, Ahmad A, Hussain A (2001). Growth of wheat seedlings raised from the grains treated with 28-homobrassinolide. Act Physiol. Plant 23:27-30. http://dx.doi.org/10.1007/s11738-001-0018-9

Hayat S, Ahmad A, Mobin M (2001a). Carbonic anhydrase, photosynthesis, and seed yield in mustard plants treated with phytohormones. Photosynthetica 39:111-114. http://dx.doi.org/10.1023/A:1012456205819

Hiscox JA, Israelstam GE (1979). A method for the extraction of chlorophyll leaf tissue without mecreation. Can. J. Bot. 57(2):1332-1334. http://dx.doi.org/10.1139/b79-163

Jones NP, Sreenivas MN (1993). Effect of inoculation of VA mycorrhiza and phosphate solubilizing bacteria on rhizosphere microflora of sunflower. II. AzotobacterAzotobacter and phosphate solubilizing bacteria. J. Ecotoxicol. Environ. Monit. 3(1):55-58.

Kaya MD, Kolsarici O (2011). Seed yield and oil content of some sunflower (*Helianthus annuus* L.) hybrids irrigated at different growth stages. Afr. J. Biotechnol. 10(22):4591-4595.

Khripach V, Zhabhinskii V, de Groot A (2002). Twenty years of brassinosteroids: steroidal plant hormones warrant better crops for the XX1 century. Ann. Bot. 86:441-447. http://dx.doi.org/10.1006/anbo.2000.1227

Lawal BA, Obigbesan GO, Akanbi WB, Kolawole GO (2011). Effect of planting time on sunflower (*Helianthus annuus* L.) productivity in Ibadan, Nigeria. Afr. J. Agric. Res. 6(13):3049-3054.

Li JM, Chory J (1999). Brassinosteroids action in plants. J. Exp. Bot. 50:275-282. http://dx.doi.org/10.1093/jexbot/50.332.275

Lopez-Valdez F, Fernandez-Luqueno F, Ceballos-Ramirez JM, Marsch R, Olalde- Portugal V, Dendooven L (2011). A strain of Bacillus subtilis stimulates sunflower (*Helianthus annuus* L.) growth temporarily. Sci. Horticult. 128:499-505. http://dx.doi.org/10.1016/j.scienta.2011.02.006

Loubser HL, Human JJ (1993). The effect of nitrogen and phosphorus fertilization on the phosphorus absorption by sunflowers. J. Agron. Crop Sci. 171(3):206-215. http://dx.doi.org/10.1111/j.1439-037X.1993.tb00132.x

Mai YY, Lin JM, Zeng XL, Pan RZ (1989). Effects of brassinolide on the activity of nitrate reductase in rice seedlings. Plant Physiol. Commun. 2:50-52.

Malakooti MJ (2000). Sustainable agriculture and yield increment by optimum fertilizer utilization in Iran",2nd edition. Agricultural Extension Publications, Iran.

Mehrvarz S, Chaichi MR, Alikhani HA (2008). Effects of phosphate solubilizing microorganisms and phosphorus chemical fertilizer on yield and yield components of barley (*Hordeum vulgare* L.). Am. Eur. J. Agric. Environ. Sci. 3(6):822–828.

Meudt WJ, Thompson MJ, Bennett HW (1983). Investigations on the mechanism of brassinosteroid response. III. Techniques for potential enhancement of crop production. In: Proceedings of the 10th Annual Meeting of the Plant Growth Regulators Society of America. Madison, USA. pp. 312-318.

Mitchell JW, Gregory LE (1972). Enhancement of overall growth, a new response to brassins. Nature 239:253-254.

Mukherjee PK, Rai RK (2000). Effect of Vesicular arbuscular mycorrhizae and phosphate solubilizing bacteria on growth, yield and phosphorus uptake by wheat (Triticum aestivum) and chick pea (*Cicer arietinum*). Indian J. Agron. 45(3):602-607.

Nakashita H, Yasuda M, Nitta T, Asami T (2003). Brassinosteroid functions in a broad range of disease resistance in tobacco and rice. Plant J. 33(5):887-898. http://dx.doi.org/10.1046/j.1365-313X.2003.01675.x

Nayak SK, Murthy KS (1980). Effect of varying light intensities on growth parameters in rice. Indian J. Plant Physiol. 23:309-316.

Oraki H, Alahdadi I, Khajani FP (2011). Sunflower (*Helianthus annuus* L.) hybrids seeds distribution modelling: Normal, lognormal and weibull models. Afr. J. Agric. Res. 6(2):618-623

Patra P, Pat BK, Ghosh GK, Mura SS, Saha A (2013). Effect of Biofertilizers and Sulphur on Growth, Yield and Oil content of Hybrid Sunflower (*Helianthus annuus* L.) in a Typical Lateritic Soil. 2:603. http://dx.doi.org/10.4172/scientificreports.603

Peix A, Mateos PF, Barrueco CR, Molina EM Velazquez E (2001). Growth promotion of common bean (*Phaseolus vulgaris* L.) by a strain of Burkholderia cepacia under growth chamber conditions. Soil Biol. Biochem. 33:1927-1935. http://dx.doi.org/10.1016/S0038-0717(01)00119-5

Prakash M, Suganthi S, Gokulakrishnan J, Sabesan T (2008). Effect of Homobrassinolide on Growth, Physiology and Biochemical Aspects of Sesame. Karnataka J. Agric. Sci. 20(1):110-112.

Ramraj VM, Vyas BN, Godrej NB, Mistry KB, Swami BN, Singh N (1997). Effects of 28-homobrassinolide on yields of wheat, rice, groundnut, mustard, potato and cotton. J. Agric. Sci. 128:405–413. http://dx.doi.org/10.1017/S0021859697004322

Rao SSR, Vardhini BV, Sujatha E (2002). Brassinosteroids- a new class of plant phytohormones. Curr Sci. 82:1239-1244.

Rodriguez H, Fraga R (1999). Phosphate solubilizing bacteria and their role in plant growth promotion. Biotechnol. Adv. 17:319-339. http://dx.doi.org/10.1016/S0734-9750(99)00014-2

Rokhzadi A, Toashih V (2011). Nutrient uptake and yield of chickpea (*Cicer arietinum* L.) inoculated with plant growth-promoting rhizobacteria. Aust. J. Crop Sci. 5(1):44-48.

Rondanini DP, Savin R, Hall AJ (2007). Estimation of physiological maturity in sunflower as a function of achene water concentration. Eur. J. Agron. 26:295–309. http://dx.doi.org/10.1016/j.eja.2006.11.001

Rose RE (1957). Techniques of determining the effect of micro-organisms on insoluble inorganic phosphates. New Zealand Journal of Science and Technology. 38:773-780.

Ruiz-Lozano JM, Azcon R (1995). Hyphal contribution to water uptake in mycorrhizal plants as affected by the fungal species and water status. Plant Physiol. 95:472-478. http://dx.doi.org/10.1111/j.1399-3054.1995.tb00865.x

Sasse JM (1999). Physiological actions of brassinosteroids. In: Brassinosteroids – Steroidal Plant Hormones, eds. Sakurai, A. Yokota, T. and Clouse, S. D., Springer, Tokyo, pp. 137-161.

Sattar MA, Gaur AC (1987). Production of auxins and gibbereline by phosphate dissolving microorganisms. Zentralbl. Mikrobiol. 142:393-396.

Seiler GJ (2007). Wild annual *Helianthus anomalus* and *H. deserticola* for improving oil content and quality in sunflower. Ind. Crops Prod. 25:95–100. http://dx.doi.org/10.1016/j.indcrop.2006.07.007

Sharafai AI, Mahmud M, Lawal AB, Abubakar IU, Mohammad SG (2006). Correlation and path coefficient analysis for growth, yield and yield components of early maturing maize (*Zea mays* L). Crop Res. 21:255-259.

Sharma AK (2002). Bio-fertilizers for sustainable agriculture". Agrobios Indian Publications.

Shinde VS (1990). Response of chickpea (*Cicer arietinum* L.) to phosphorus with and without PSB (Microphos) as influenced by applied sulphur. Ph.D. Thesis, IARI. Division of Agronomy, New Delhi.

Sieverding E (1991). Vesicular- Arbuscular Mycorrhiza management in Tropical Agrosystem. Deutsche Gesellschaft fur Technische Zusammenarbeit, Eschborn.

Sinclair TR, Hoffer RM Schreiber MM (1971). Reflectance and internal structure of leaves from several crops during a growing season. Agron. J. 63:864-868. http://dx.doi.org/10.2134/agronj1971.00021962006300060012x

Singh S, Kapoor KK (1994). Solubilization of insoluble phosphates by bacteria isolated from different sources. Environ. Ecol. 12:51-55.

Smith SE, Robson AD, Abbott LK (1992). The involvement of mycorrhizas in assessment of genetically dependent efficicncy of nutrient uptake and use. Plant Soil 146:169-179. http://dx.doi.org/10.1007/BF00012010

Vardhini BV, Rao SSR (1998). Effect of brassinosteroids on growth, metabolite content and yield of Arachis hypogaea. Photochemistry 48:927-930. http://dx.doi.org/10.1016/S0031-9422(97)00710-3

Yadav SP, Shrivastava UK (1997). Response of chickpea (*Cicer arietinum*) to phosphorus and biofertilizer. Legume Res. 20(2):137-140.

Yang ZS, Shi GA, Jin JH (1992). Effects of epibrassinolide, a growth promoting steroidal lactone I. Activity in selected bioassays. Phsiol. Plant 53:445-452.

Zubillaga MM, Aristi JP, Lavado RS (2002). Effect of phosphorus and nitrogen fertilization on sunflower (*Helianthus annuus* L.) nitrogen uptake and yield. J. Argon. Crop Sci. 188:267-274. http://dx.doi.org/10.1046/j.1439-037X.2002.00570.x

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