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Full Length Research Paper

Application of GIS in pig production system in Nigeria

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This study aimed to identify the pig producers across various administrative regions and to determine the pig production system in Nigeria through an intensive survey of pig farms with the use of Global Positioning System (GPS). Categorization of farms by proportion in Nigeria showed that 81.1% of states had very low (<5) and low (5 to 50) number of pig farms, 8.1% of states had medium (50 to 100) number of pig farms and 10.8% of states had high (100 to 150) and very high (>150) number of pig farms. In Nigeria, there are two separate pig production systems: small and medium scales. The small scale system is essentially characterized by very low to low scale of production. Analysis of categories of production in Nigeria showed that 45.9% of states recorded very low (<100 pigs) and low (100 to 1,000 pigs) scales of production, 46% of states were in the medium (1,000 to 10,000 pigs) scale of production and 8.1% of states had high (10,000 to 100,000 pigs) and very high (>100,000 pigs) scales of production. GIS qualitative analysis showed that in the North, the production classes: very low, low, medium, high and very high scales of production by states were 40, 30, 25, 0 and 5%, respectively while in the South, the production classes were 0, 17.6, 70.6, 11.8 and 0%, respectively. A high number of Northern states were identified in the low category of pig production scales while most Southern states were in the medium category. The application of GIS revealed that the pig sector in Nigeria is dominated by small and medium scales of production while the harsh climate and its future change pose a major threat to pig farming in the Northern Nigeria.

Key words: Data integration, extension services, farm survey, pig meat, qualitative analysis and rainfall distribution.

INTRODUCTION

The importance of livestock sub-sector is in line with recommendation of the Food and Agricultural Organization (FAO) (2003) that on an average basis, a man's daily protein intake should be between 65 to 72 g and 53% (about 35 g) of this should be animal based.

The major sources of animal protein in Nigeria are beef, pork, poultry, goats, fish and game animals (Ajala, et al., 2007). Pigs have some unique advantages over other domestic animals. They grow at a faster rate and are more prolific than cattle, sheep and goats (Holness,

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1991 and Osaro, 1995). In addition, the pig sector provides employment and generates income.

Dwindling profit in a pig enterprise has been reported to be a function of poor quality feeds resulting from unbalanced ration (Adesehinwa and Ogunmodede, 1995). However studies on pig and poultry industries seem to reveal that the initial enthusiasm in these enterprises, especially pig production, is being constrained due largely to dwindling profit margins (Ogunfowora, 1980).

Evidences abound in different parts of the world that pig sector is moving forward, unlike what is obtainable in Nigeria. According to Pig International (1997), a single integrated Spanish Company "VALL Company of Spain" hit a production target of two million seven hundred thousand pigs, as early as 1986, with directors still making projection of making it four million by the year 2002. A similar report was given about farmers choice in Kenya, a company that single handedly produces virtually all the national herd of sows in Kenya, having 2500 sows in contract and another 2000 from internal production (Pig international, 1999). It is however pathetic to compare all these figures with the meager pig population system in Nigeria. The large Muslim population of northern Nigeria may also not favour profitable pig production in that part of the country (Adetunji and Adeyemo, 2012).

In Nigeria, the Pig sector is dominated by small scale and medium scale holders. According to Eusebio (1980), backyard pig farming and large scale pig production are more profitable than medium scale pig production. His claim was that large scale pig producers enjoy economies of scale which lowers the unit cost of production when compared to small scale of production. He further claimed that the cost of feeding is skipped. He discouraged medium scale production for the reason that purchasing of feed ingredients were made in small quantities. It is generally known that the more the quantity of production the more the profit. Ojo (2000) said that the problem associated with small scale farming was that their scale of operation made it for them to obtain loan due to lack of collaterals. Economic development is normally accompanied by improvements in a country's food supply and the gradual elimination of dietary deficiencies, thus improving the overall nutritional status of the country's population. Furthermore, it also brings about qualitative changes in the production, processing, distribution and marketing of food (WHO, 2003). In spite of all policies that successive government made, the prevailing economic hardship in Nigeria has also made the pig production system to remain underdeveloped as a result of the poor purchasing strength of the consumers. Generally, livestock production in Nigeria is not as efficient relative to the developed countries. Apart from poultry, pig farming is a class of animal production that is not subjected to such heavy losses resulting from failure to follow good sanitation practices. Despite the inherent productive capabilities of pigs, its production is low in Nigeria and it is faced with a number of problems amongst which are inadequate supply of feed, water, worm infestation, good health management, religious, veterinary services, change in climate, housing and waste disposal, as well as government policy.

Social factors that could influence pig production in Nigeria include a general preference for ruminant meat and lack of incentives for investing in large scale pig production due to economic, religious, political and climatic factors. The large Moslem populations of Northern Nigeria may also not favour profitable pig production in that part of the country (Adetunji and Adeyemo, 2012). Other social factors that have militated against pig production in Nigeria include the belief by the general populace that pigs are dirty and constitute a health hazard. This is absolutely untrue for pigs that are produced under modern intensive production techniques since under suitable modern husbandry pigs can be very clean animals (Ajala et al., 2007.) It is relatively easy to establish intensive pig production in a developing country like Nigeria if capital are available and adequate feed supplies are assured (Ogunniyi and Omoteso, 2011). Inadequate supply of feed poses the most critical problem. A number of pig farmers are faced with this problem leading to heavy losses due to malnutrition's and increased death of embryo during early stage of pregnancy. Hence, feed determines both productivity and growth performance of livestock. It has been identified as a major constraint in animal production. Consequently, animal farmers opt for small scale production mainly to reduce cost of feeding. There are indications that pig production in the study area is mostly in the hands of small scale producers who may not have access to credit facilities because generality of the pig farmers are assumed to be peasants. The social factors that have militated against pig production in Nigeria include the belief by the general populace that pigs are dirty and constitute a health hazard. This is absolutely untrue for pigs that are produced under modern intensive production techniques since under suitable modern husbandry pigs can be very clean animals (Ajala et al., 2007.). Therefore this study is expected to provide relevant information that would encourage pig farmers and new entrants to venture into pig farming, as Nigerian population provides a readily available market.

As the genetic potential of pigs is being improved, management intervention is also essential to help overcome the constraints on production set by variation in climate. The physical environment and the health hazards also pose threat to huge production of pigs in Nigeria. Heat stress is more common in the dry season and especially when the environmental temperature and relative humidity are high with prolonged exposure to direct sunlight (Okoruwa, 2014). There is little or no robust data on the current facts about entrepreneurial characteristics and constraints to the development of pig

Figure 1. Spatial distribution of pig farms in the study area.

enterprises in Nigeria. Previous research involving the effects of heat stress on reproduction has been conducted using dairy cows (West, 2002). Amundson et al. (2006) also reported that of the environmental variables studied, minimum temperature had the greatest influence on the percent of cows getting pregnant. With all clarity, increases in temperature and/or humidity in the tropical regions of Nigeria have the potential to affect and reduce conception rates of pigs not adapted to those conditions especially in the Northern region of the country. If farmers are not making efficient use of existing technology, low production will be recorded both in food and livestock (Ajibefun and Daramola, 2003). This study will definitely provide succinct and invaluable information to policy makers, to help them design policies that will improve the pig sector as a control in filling the identified gaps in food security in Nigeria. There is a continual need therefore to generate information on the facts about pig production and such information is needed for proper planning and regulation of the sector in Nigeria. In this view, application of Geographic Information Systems (GIS) technology is needed to collect data, store, manage, analyse and produce useful information for timely monitoring of the pig sector in Nigeria. GIS capability in pig farming is achievable and useful in production level scaling, monitoring feed cost, investigating credit facility sources and disease spread analysis.

MATERIALS AND METHODS

Pig farm survey

The survey was carried out in the year 2010 across all geopolitical zones in Nigeria. The spatial dataset of identifiable pig farms were collected by surveyors with the use of GPS for the identification of positions of the farms in the study area (Figure 1). The data collected was accompanied by the administration of a set of questionnaire which was designed to obtain information on the production level of the sampled farms. The farm survey stage is the most important stage as its accuracy ascertains the production capacity estimate for the investigated boundaries.

GIS data integration

For the purpose of data integration and querying, the logical structuring of the spatial and attribute data of both the farm and questionnaire were performed using ArcGIS ® capabilities. The geographical position (centroid) of each farm was defined as a feature data and linked with the attribute dataset of the respective farm to derive the farm production in a GIS environment. The production scales of the farms resulted in the local to the national estimates of scales of production. The framework of the mapping procedure as adopted from Omodele and Okere (2014) is shown in Figure 2.

RESULTS AND DISCUSSION

Analysis of production by states

Table 1 displays the production by States in Nigeria. No records of pig farms and their production were made available for Jigawa, Kano, Katsina, Plateau, Yobe and Zamfara States. Bauchi had some farms record but no production data.

Analysing Table 1, the order of production by farms was profiled in Figure 3. Nassarawa State was discovered to have recorded the highest percentage production of pigs in Nigeria followed by Abia state, which had an appreciable level of production when compared with its percentage proportion of farms. Enugu state was also discovered to have recorded a significant level of production. It was expected that Kaduna state which had

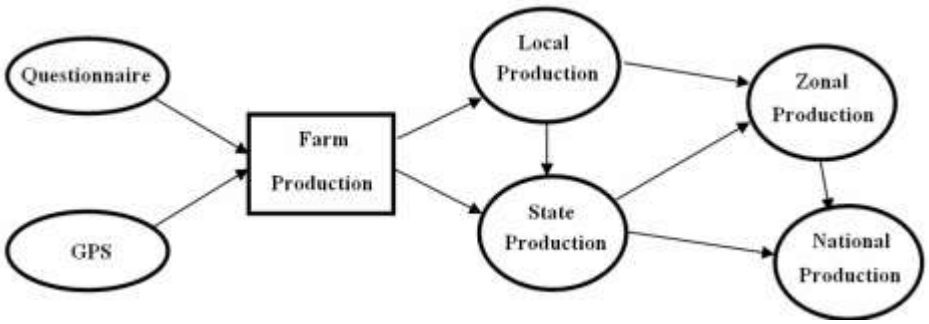

Figure 2. Adopted mapping procedure from local to the national level.

Table. 1. Pig production status per State in 2010 (pigs).

S/N	State	No of farms	Production
1	Abia	32	19235
2	Adamawa	92	3026
3	Akwa Ibom	108	4589
4	Anambra	40	5535
5	Bauchi	12	0
6	Bayelsa	9	1151
7	Benue	29	6836
8	Borno	15	225
9	Cross River	16	472
10	Delta	33	3390
11	Ebonyi	30	4992
12	Edo	45	3674
13	Ekiti	27	1798
14	Enugu	105	14659
15	Federal Capital Territory	5	102
16	Gombe	40	2466
17	Imo	27	4955
32	Jigawa	0	0
18	Kaduna	157	4857
33	Kano	0	0
34	Katsina	0	0
19	Kebbi	14	405
20	Kogi	9	615
21	Kwara	12	633
22	Lagos	14	2961
23	Nassarawa	122	104149
24	Niger	13	789
25	Ogun	72	5740
26	Ondo	30	927
27	Osun	66	4890
28	Oyo	36	6700
35	Plateau	0	0
29	Rivers	7	552
30	Sokoto	1	66
31	Taraba	33	5192
36	Yobe	0	0
37	Zamfara	0	0

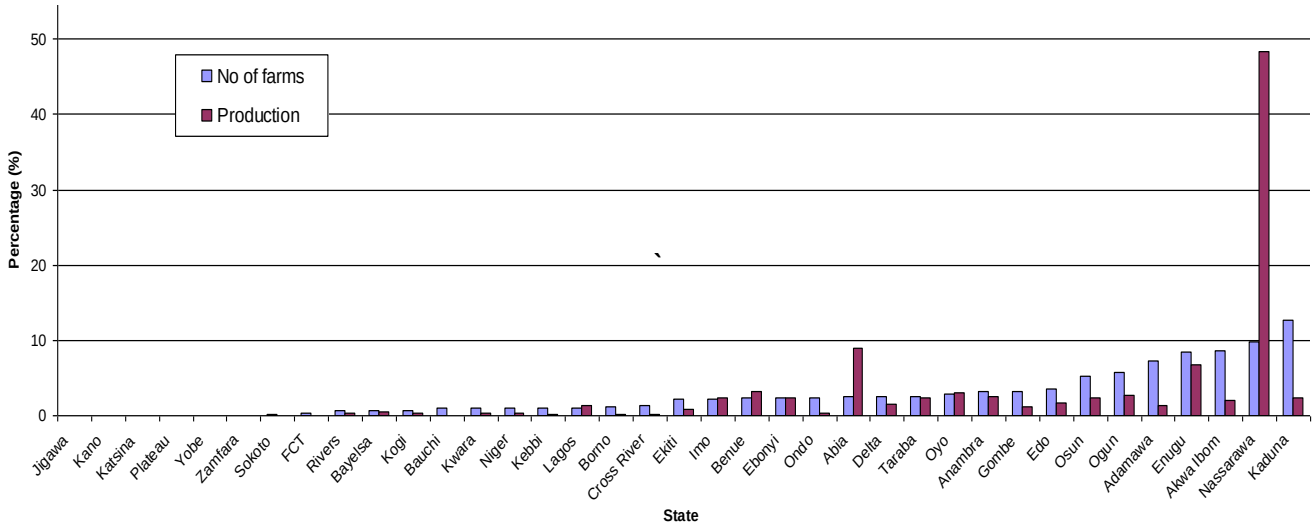

Figure 3. Percentage production per state in Nigeria.

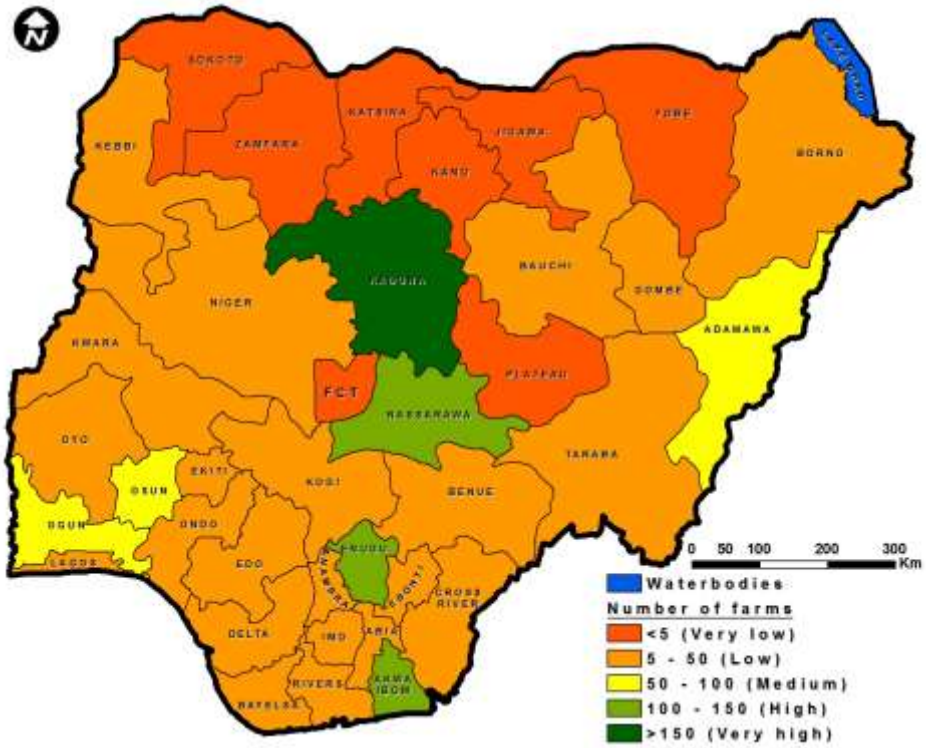

Figure 4. Pig farms proportion by States in Nigeria.

the highest number of farms in Nigeria should have the highest proportion of pigs but this presumption was not achieved. The occurrence in Kaduna state justifies the finding of Omodele et al. (2014) that the high number or proportion of farms in an area does not guarantee a high production in such a locality.

State-based analysis of farms proportion and production

As displayed by Figure 4 and expressed in Figure 5, categorization of farm proportion in the states in Nigeria showed 21.6% of states had very low (<5) proportion of

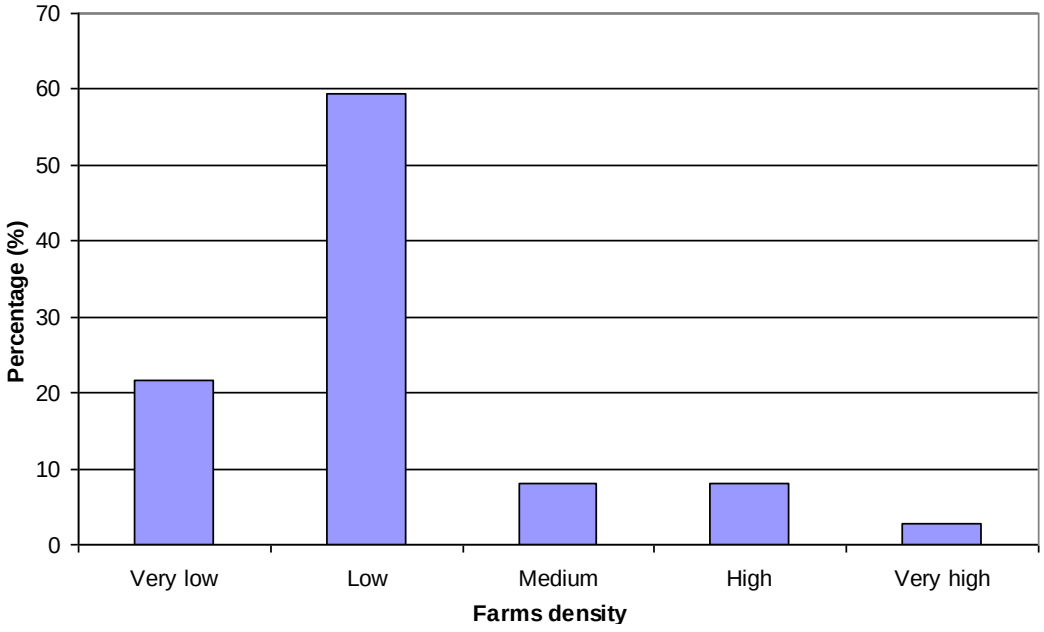

Figure 5. Categories of percentage proportion of pig farms in Nigeria.

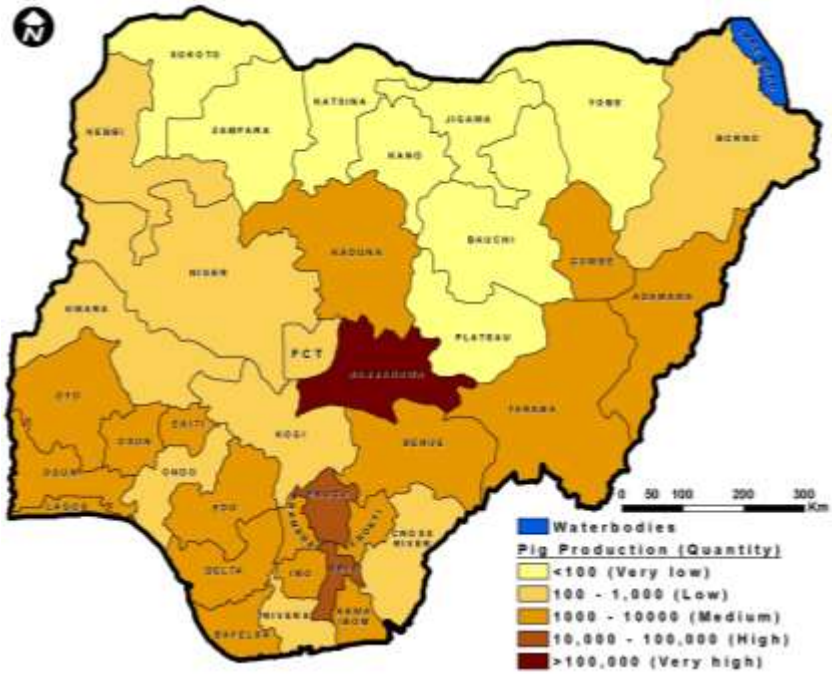

Figure 6. Pig production level by States in Nigeria.

farms, 59.5% of states had Low (5 to 50) proportion of farms, 8.1% of states had medium (50 to 100) proportion of farms, 8.1% of states had high (100 to 150) proportion of farms and 2.7% of states had very high (>150) proportion of farms. The highest proportion or density of pig farms was discovered in the low density category. This connotes that pig farms are not many in Nigeria, therefore pig production level is expected to be low because it is expected that the lower the proportion of farms the lower the quantity of production. By analyzing the categories of production in the states as shown in Figure 6 and summarized by Figure 7, the analysis showed 21.6% of states in the very low (<100 pigs) production class, 24.3% of states in the low (100 to 1,000

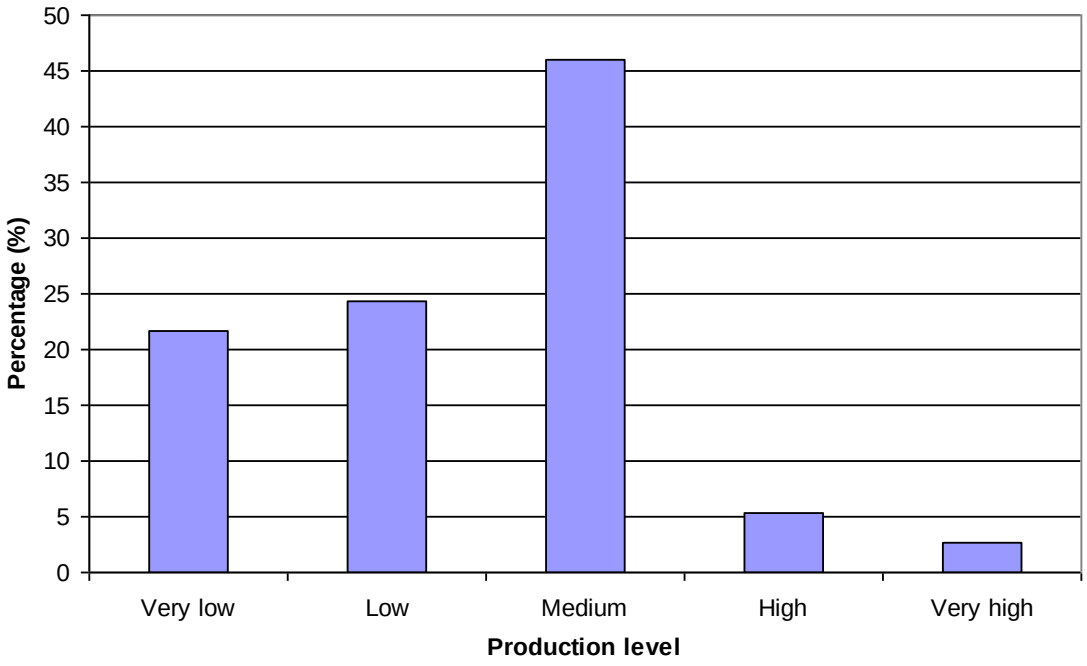

Figure 7. Categories of pig production in Nigeria.

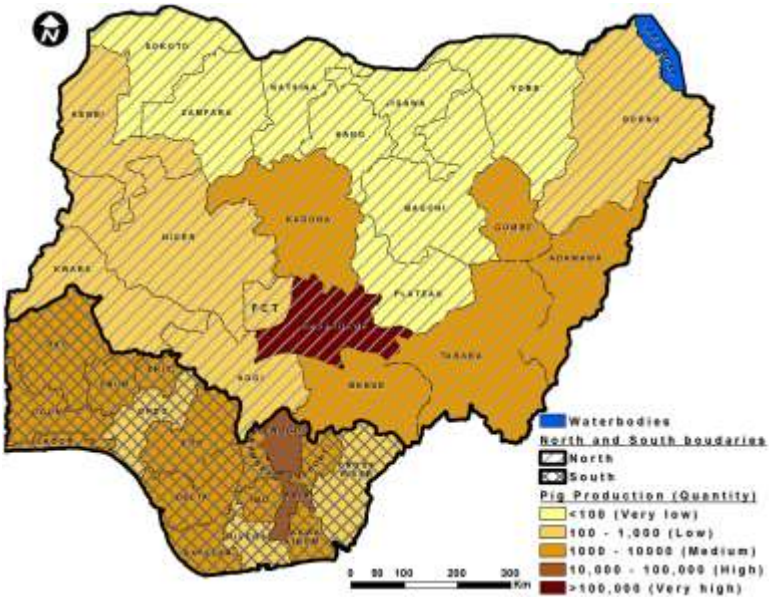

Figure 8. North and South assessment of pig production.

pigs) production class, 46% of states in the medium (1,000 to 10,000 pigs) production class, 5.4% of states in the high (10,000 to 100,000 pigs) production class and 2.7% of states in the very high (>100,000 pigs) production class. The production analysis showed that the highest pig production level in Nigeria is in the medium class. This indicated that the pig sector and its production systems in Nigeria are absolutely in the small and medium scales.

North and South qualitative production analysis

As displayed in Figure 8 and summarized in Figure 9, further analysis of the qualitative performance of states in the Northern and Southern zones showed that in the

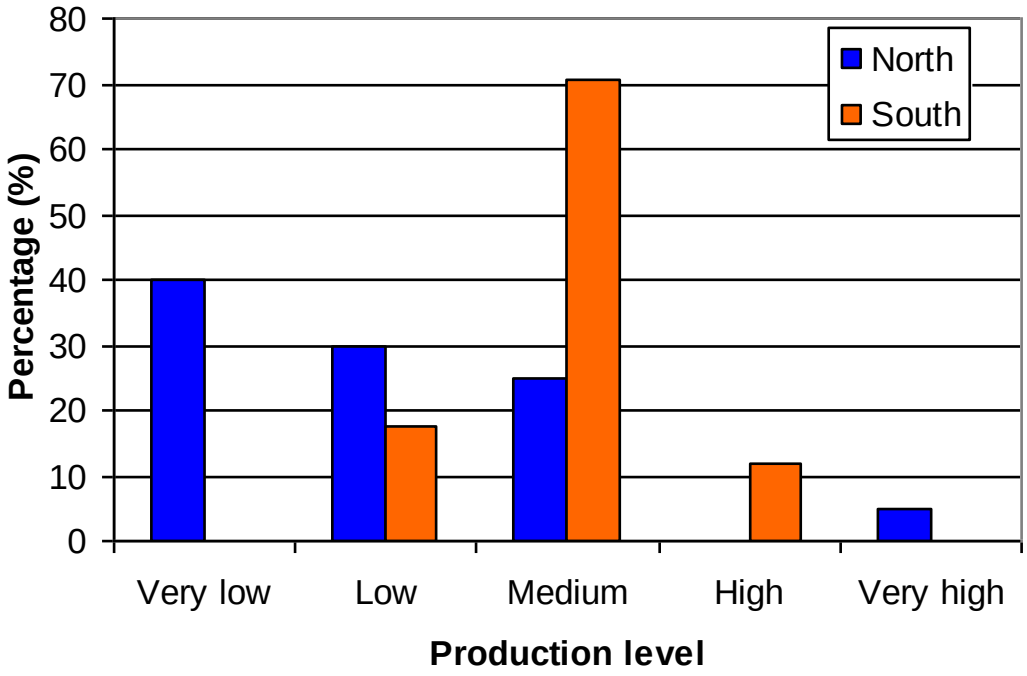

Figure 9. North and South pig production level.

North the production classes: very low, low, medium, high, very high were 40, 30, 25, 0 and 5%, respectively while in the South, the production classes were 0, 17.6, 70.6, 11.8 and 0%, respectively. The most practiced production classes of pig meat by States in the North were in the low categories. In the South, the widely practiced class was the Medium scale of production. There existed an appreciable acceptance and general production of pig meat in the Southern part of Nigeria as compared with the Northern part. This approach has formed a platform on which further research could be made.

Climatic approach to pig production

As climatic conditions vary across geographic boundaries in Nigeria, the surrounding environmental conditions are expected to directly affect the rates of heat gain or loss by all animals. Lack of prior knowledge of adequate conditioning to weather events most often results in these catastrophic losses. As noticed that the atmospheric temperature of the earth has been increased as a result of cumulative effects of greenhouse gases emitted into the atmosphere through different industrial and agricultural activities of human. The performance of both domestic and wild animals ranging from insects, amphibians, birds to mammals are expected to be affected by climate variation. Therefore rainfall distribution pattern plays a crucial role in this regard. As in Figure 10, the Southern part of Nigeria experiences a cooler atmospheric condition from the annual rainfall distribution. Adopting the rainfall distribution patterns: <1000mm, (1000-1400)mm, (1401-1700)mm, (1701-2000)mm and >2000mm, Table 2 and Figure 11 showed that 5% of farms within <1000mm of rainfall had 0.8% of production, 46.2% of farms within (1000-1400)mm of rainfall had 69.9% of production, 14.3% of farms within (1401-1700)mm of rainfall had 0.6% of production, 15.3% of farms within (1701-2000)mm of rainfall had 11.5% of production and 19.2% of farms within >2000mm of rainfall had 17.2% of production. The extreme part of the Moslem communities in the North with the lowest rainfall distribution pattern had the lowest farms proportion and production. Apart from the religious belief/barrier, the harsh climate in the North poses another major threat to pig farming in that part of the country.

CONCLUSION AND RECOMMENDATION

This study with the use of GIS techniques has revealed that the pig sector in Nigeria is dominated by small scale and medium scale holders. A high number or proportion of farms in an area does not guarantee a high production in such a locality or zone. There existed an appreciable acceptance general acceptance in general production of pig meat in the Southern part of Nigeria where a higher number of states had medium scale of pig production. A State and region-based agricultural development and monitoring of piggery is essential as it encourages grass-root awareness and sensitization, especially in the

forgotten pig meat production sector. Introduction of modern hygienic practices could reduce the major constraints of religious and cultural restrictions in pig farming in Nigeria. Pig production is essential to food security in providing a fast and adequate protein level for the rapidly growing Nigerian population. As the climate changes, substantial funding of the sector is essential especially for construction of modern housing system in the tropical regions of Nigeria. Therefore, application of GIS techniques has assisted the decision makers and strengthened their horizon and perception of the pig farming system in Nigeria. GIS has also revealed the areas where possible developmental strategies, agricultural extension services and further research are most essential in the pig farming domain of Nigeria.

Conflict of interests

The authors have not declared any conflict of interest.

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REFERENCES

Adesehinwa AOK, Ogunmodede BK (1995). Swine feeds and practical Feed Composition techniques in National pig production Training manual N.A.E.L.S/ABU. pp. 27-56.

Adetunji MO, Adeyemo KE (2012). Economic Efficiency of Pig Production in Oyo State, Nigeria: A Stochastic Production Frontier Approach. American Journal of Experimental Agriculture 2(3):382-394.

Ajala MK, Adesehinwa AOK, Mohammed AK (2007). Characteristics of small-holder pig production in southern Kaduna area of Kaduna state Nigeria: American-Eurasian Journal of Agricultural and Environmental Sciences 2(2):182-188.

Ajibefun IA, Daramola AG (2003). Efficiency of microenterprises in the Nigerian economy. AERC Research Paper 134 African Economic Research Consortium Nairobi. Available at: http://dspace.africaportal.org/jspui/bitstream/123456789/32113/1/rp134.pdf?1

Amundson JL, Mader TL, Rasby RJ, Hu QS (2006). “Environmental effects on pregnancy rate in beef cattle”, Journal of Animal Science 84:3415-20.

Eusebio JA (1980). Pig production in the Tropics, Longman group Limited Essex U. K.

FAO (2003). Food and Agricultural organization of the United Nations, Quarterly Bulletins of Statistics.

Holness HD (1991). The tropical Agriculturist (pigs) publ. by Macmillan/CTA Ltd, London.

Ngoka DA (1979). Principles of Livestock Production in the Tropics, Alphabet Publishers, Owerri. pp. 1-67.

Ogunfowora O (1980). An Evaluation of the state of Developed and Economics of pig production in Nigeria. Report prepared for the Federal livestock Department Lagos, Nigeria.

Ogunniyi LT, Omoteso OA (2011). Economic Analysis of Swine Production in Nigeria; A Case Study Of Ibadan Zone Of Oyo State. Journal of Human Ecology 35(2):137-142.

Ojo SO (2000). Enhancing livestock production in Nigeria Book of proceeding of the 25th Annual NSAP Conference 19-23 March 2003, Umudike.

Okoruwa MI (2014). Effect of Heat Stress on Thermoregulatory, Live Bodyweight and Physiological Responses of Dwarf Goats in Southern Nigeria. European Scientific Journal 10(27):255-264.

Omodele T, Okere IA (2014). GIS application in poultry production: identification of layers as the major commercial product of the poultry sector in Nigeria. Livestock Research for Rural Development, Volume 26, Article #097. Available at: http://www.lrrd.org/lrrd26/5/omod26097.html.

Omodele T, Okere IA, Deinne CE (2014). Technical assessment of rural development of the poultry meat sector in Ogun State, South Western Nigeria: A Geographic Information System (GIS) approach. Livest. Res. Rural Dev. 26(7).

Osaro OM (1995). Enhancing production performance of small-holder pig farmers In: pig production workshop Training manual NAERLS, ABU, Zaria, Nigeria. pp. 100-130.

Pig international September (1997). Europe’s Biggest produce. 27(9)57-61.

Pig International (1999). Kenya’s Dominants integrator. 29(12):26-30.

West JW (2002). Physiological effects of heat stress on production andreproduction.Pages1–9inProc.Tri-StateDairyNutr.Conf., Fort Wayne, IN.

WHO (2003). Diet, Nutrition and the Prevention of Chronic Diseases. WHO Technical Report Series 916:13.

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Composting coffee pulp with Minjingu phosphate rock improves phosphorus availability for tomato uptake

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A glasshouse experiment was conducted to evaluate the effect of composting coffee pulp with phosphate rock on phosphorus (P) availability for plant uptake. Coffee pulps composted with or without phosphate rock and Minjingu phosphate rock applied alone were evaluated as source of P for tomato growing on a Chromic Acrisol. All P sources were applied at varying rates of 0, 20, 40, 60, 80 and 100 mg P kg^{-1} and all other limiting nutrients were adjusted to recommend levels using industrial fertilizers and/or reagent grade laboratory salts. Phosphorus uptake of tomato plants receiving coffee pulp composted alone, Minjingu phosphate rock alone and coffee pulp composted with Minjingu phosphate rock increased 11, 13 and 18 times above the control. Observed P concentrations in tomato plants receiving external P sources were 23, 36%and 110% of the concentrations in control plants. Composting coffee pulp with Minjingu phosphate rock was concluded as a potential technique for improving phosphorus availability and uptake by tomato.

Key words: phosphate rock, coffee pulp, phosphate rock enriched compost, tomato, phosphorus uptake.

INTRODUCTION

Most of the tropical agricultural soils are highly weathered, leached and consequently acidic. These soils are highly deficient in phosphorus (P) thus require substantial P inputs for optimum crop production (Buehler et al., 2002). Phosphorus deficiencies are mainly corrected via application of water soluble P fertilizers and manures. However, water soluble P fertilizers are of limited availability and unaffordable to most small holders in developing countries (Kpomblekou and Tabatabai, 2003; Mowo et al., 2006)

Farmyard manure is similarly not readily available for most smallholder crop producers and where available is of poor quality (Buresh et al., 1997). Continuous cropping associated with low levels of fertilizers and manure applications results into soil degradation and subsequently season after season decline of small holder crop productivity.

Promoting the use of locally available Minjingu phosphate rock (MPR) has been considered as the most promising alternatives to the expensive mineral P fertilizers in Tanzania. However, slow dissolution of the MPR results into limited availability of P for plant uptake, hence limited first season crop response to applied MPR. Production of phosphate rock enriched composts has been reported as an alternative way for improving phosphorus release from low value phosphate rocks and

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crop response to applied PRs (Ditta et al., 2017; Meena and Biswas, 2015; Mihreteaba et al., 2015; Ikerra et al., 2006). Availability of P for plant uptake from PR enriched compost is thus a product of the quality of composted plant biomass and the PR used to enrich the compost. High quality plant biomass is therefore a prerequisite for enhancing P availability from PRs through composting.

Coffee pulp is a readily available crop residue in all the coffee producing areas of the world, including Tanzania. Its direct application as a soil amendment in coffee fields is restricted by the presence of toxic compounds like phenols, local heat generation and coffee berry disease spread risks (Pandeya et al., 2000; Preethu et al., 2007). Different technologies have been applied to manage coffee pulp for the purpose of minimizing the challenges associated with direct application of coffee pulp and environmental pollution risks. Reported technologies include composting coffee pulp and using as a growing media in greenhouse crop production (Berecha et al., 2011); soil application of composted pulp as a fertilizer material or soil conditioner (Dzung et al., 2013); using coffee pulp as feedstock in heat energy or biogas production (Cubero-Abarca, et al. 2014); as well as using dried coffee pulp as supplement in ruminant diets (Núñez et al., 2015). Although application of composted coffee pulp as soil organic amendment is documented, the effect of composting coffee pulp with PRs on P release and availability for plant uptake is still unknown. This study was therefore based on this knowledge gap and evaluated the effect of composting coffee pulp with MPR on P availability and uptake by tomato.

MATERIALS AND METHODS

Coffee pulp collection and characterization

The coffee pulp was collected from Malonji village primary processing center in Mbozi District and transported to the Tanzania Coffee Research Institute, Mbimba station for composting. Fifteen random samples were taken from a pile of fresh coffee pulp mixed into a homogenous representative sample, weighed and dried to constant weight at 60°C. The oven dried sample was ground to pass through a 0.5 mm sieve for laboratory total analysis. The moisture content, pH, total organic carbon, total N, P, Ca, and K in the coffee pulp were determined following the procedures described by Okalebo et al. (1993).

Compost preparation

Coffee pulp was composted in pits both alone and mixed with MPR. Four pits (3x2x1 m^3 each) were opened and the floor of each pit was lined with polythene sheets to avoid underground seepage. One tone of fresh coffee pulp at 70% moisture content (equivalent to 300 kg air dry weight) were mixed with 100 kg of air dry surface soil to get 400 kg of air dry mixture which was filled into the first pit. Another portion of 400 kg air dry mixture prepared in the same fashion was mixed with 100 kg of MPR (4:1) (FAO, 1987) on air dry weight basis to fill the third pit. The two pits 1 and 3 were covered with banana leaves followed by a layer of dry soil while pits 2 and 4 remained empty for the turning of the compost materials. Composting lasted for 4 months with turning of material at 4 weeks intervals to allow optimum aeration. Water was sprinkled onto the compost materials at every turn to maintain the moisture around 60% for optimum microbial activity (Graves and Hattemer, 2000).

Compost sampling and analysis

At the end of the composting period, ten random samples were taken from each of the two composting sets, homogenized and reduced by quartering into two separate 0.5 kg representative samples. Moisture content of the representative compost samples was determined following the procedures described by Graves and Hattemer (2000). Thereafter, the samples were air dried ground and sieved through 2 mm sieve for determination of the pH, total organic carbon, nitrogen, phosphorus, potassium and calcium following the standard procedures compiled by Okalebo et al. (1993).

Soil sampling and analysis

Soil sampling was done at Magadu farm located on the western part of SUA Main campus in Morogoro Tanzania. The process was preceded by a preliminary survey to identify an area with a Chromic Acrisol that has neither been under cultivation nor received any fertilizer or manure treatment for the past ten years. Ten representative surface samples (0-20 cm), 50 kg each were randomly collected and thoroughly mixed to constitute a representative composite soil sample. The soil was air dried and sieved through 8 mm for the pot experiment. One kilogram representative sample was drawn from the entire soil reduced by the quartering procedure to 0.5 kg ground and sieved through 2mm for laboratory analysis.

The Chromic Acrisol was analyzed for physical and chemical properties in the Department of Soil Science laboratory, at Sokoine University of Agriculture, Morogoro Tanzania. Particle size distribution was determined by the hydrometer method (Gee and Bauder, 1986) while pH was electrometrically determined in 1:2.5 (soil: water) suspension (Mclean, 1982). Organic carbon was determined by the Walkley and Black method (Nelson and Sommers, 1982). Total N was determined by the micro Kjeldahl method (Bremner, 1996). Available P in the soils was extracted by the Bray 1 procedure (Kuo, 1996). CEC was determined by the ammonium acetate saturation method (Rhodes, 1982). Exchangeable bases, that is Ca^{2+} and Mg^{2+} in the CEC determination filtrates (NH_4-acetate filtrates) were determined by atomic absorption spectrophotometer, while K and Na were determined by the flame photometer method (Thomas, 1982). Plant extractable Cu, Zn, Mn and Fe were extracted by DTPA and measured by atomic absorption spectrometer (Lindasy and Norvell, 1978).

Glasshouse pot experiment

A glasshouse pot experiment was carried out at the Sokoine University of Agriculture (SUA), Morogoro Tanzania. The experiment was arranged in a 3 x 8 completely randomized block design using three different P sources (CP, MPR and CPMPR) all applied at 0, 20, 40, 60, 80 and 100 mg P kg^{-1} in three replicates. Following application of all P sources; the soils were equilibrated with two liters of water/pot for 24 h to bring the soil moisture to field capacity before sowing the seeds. After 24 h of equilibration five tomato seeds were sown in each pot and thereafter, soil moisture content was maintained around field capacity by replacing equivalent amount lost through evapotranspiration. All emerging weeds were uprooted to keep weed competition at minimum level

possible. Thirty days after planting, two seedlings were thinned out leaving three seedlings per pot followed by the second split of nitrogen uniformly applied to all pots except absolute controls.

Quantification of dry matter yield and nutrient uptake

Sixty days after planting, two seedlings were harvested from each pot by cutting all above ground parts at the soil surface for the determination of dry matter yield and nutrient uptake. Harvested plants were cleaned using distilled water and oven dried to constant weight at 55°C for 72 h, and weighed using a chemical balance. After weighing, dry plant samples were chopped into small pieces then ground using a motor and pestle into a fine powder to pass through 0.5 mm sieve. A 0.5 g of fine ground sample was digested by the H_2SO_4 - H_2O_2 and HNO_3 - H_2O_2 procedures and the digests were analyzed for N and P, K, Ca and Mg contents respectively, following procedures compiled by Okalebo et al. (1993).

Data analysis

Dry mater yields, P uptake and plant tissue P concentration data was subjected to analysis of variance (ANOVA) and the means were separated at P ≤ 0.05using Duncan's New Multiple Range Test.

RESULTS AND DISCUSSION

Physico-chemical properties

Selected physico-chemical properties of the soil used is as shown in Table 1.

Properties of the chromic acrisol

Selected properties of the surface (0-20 cm) Chromic Acrisol analyzed before applying P sources were as presented in Table 2. The soil pH was low (< 5.5) with a very strong acid reaction and very low level of organic carbon (< 4%) and Bray I extractable phosphorus (< 7 mg kg^{-1} soil) (Landon, 1991). Low pH could be attributed to the nature of the parent material (acidic parent material), extensive weathering, loss of basic cations and anions through the processes of plant uptake and most probably due to leaching. Low levels of extractable P on the other hand could be attributable to the low inherent P in the soil's parent material and transformation of plant available P into unavailable Fe –P and Al-P to of P as influenced by Fe^{3+} and Al^{3+} .

Exchangeable K is categorized as < 0.2 cmol (+)/kg (low), 0.2-0.4 cmol (+)/kg (medium) and > 0.4 cmol (+)/kg (high); exchangeable Ca < 1 cmol (+)/kg as moderately low, 1-2 cmol (+)/kg as moderately high and 2-3 cmol (+)/kg as high. Critical values of Mg on the other hand are categorized as ≤ 0.2 cmol (+)/kg (low), 0.2-0.5 cmol (+)/kg (medium) and > 0.5 cmol (+)/kg (high). Based on this categorization, the Chromic Acrisol requires substantial N, P, K and Ca inputs for successful tomato production. Its pH was less than the optimum pH range of 6.0 to 7.0 recommended for successful tomato production thus requires liming. The use of MPR and composts rich in Ca was thus expected to improve the soil pH and this would be as a secondary effect to plant nutrient supply.

Properties of Minjingu phosphate rock

Selected chemical properties of the Minjingu phosphate rock **(**MPR) used in the study are as presented in Table 3.

The concentration of total P is high enough (> 5%) thus MPR qualifies as a fertilizer based on the criteria set by FAO (2000). However, Ca content of MPR is also high implying that P exists as Ca-P which is a complex form not easily released for plant uptake. Direct application of MPR as P fertilizer is therefore only feasible in acidic soils with large number of Ca sinks where MPR could have some liming effects as well.

Properties of composts used in the study

Selected properties of compost materials used in the study were as presented in Table 3.

The moisture content of the coffee pulp composted alone was slightly lower than that of the coffee pulp composted with MPR (26.42 and 29.08%, respectively). Differences in moisture contents of the two composts were attributed to minor differences in drainage of the composting pits. Graves and Hatteemer (2000) recommended 30-40% moisture content to be the optimal for finished or mature composts ready for soil application. Based on this criterion, both CP and CPMPR had moisture contents close to the recommended moisture content range. Minor difference in moisture contents could not have significant effect on tomato plant response since all soils were equilibrated to field capacity moisture content before sowing the tomato seeds and irrigation water was applied to maintain moisture content at field capacity.

High pH_w for CP and CPMPR was due to high contents of basic cations (Ca, K and Mg) in the raw materials (coffee pulp and MPR) used to produce the composts. Both CP and CPMPR had high organic carbon contents (46.9 and 38.3% respectively) due to elevated carbon contents of coffee pulp used as raw materials for the compost production. However, the two composts had C: N ratio within the range ≤ 30 recommended by Graves and Hatteemer (2000) for successful mineralization of organic materials in soils. Total P and Ca contents were higher in CPMPR than CP suggesting its higher contents in MPR than coffee pulp while total K was in the reverse order for the two composts suggesting the effect of higher K contents in coffee pulp than was in MPR.

Table 1. Selected physico-chemical properties of the soil used.

Soil property	Mean value	Rating	Rating reference
pH (H_20)	4.30	Low	Landon (1991)
Organic carbon (%)	1.10	Very low	Landon (1991)
Total N (%)	0.11	Low	Landon (1991)
Total P (%)	0.10	Low	Dierolf et al. (2001)
Bray I P ($mgkg^{-1}$)	5.59	Low	Landon (1991)
Exchangeable K (cmol(+)/kg)	0.35	Medium	Landon (1991)
Exchangeable Ca (cmol(+)/kg)	1.45	Low	Landon (1991)
Exchangeable Mg (cmol(+)/kg)	1.69	High	Landon (1991)
CEC (pH 7)	16.80	Medium	Landon (1991)
DTPA extractable Cu (mg/kg)	4.64	High	Landon (1991)
DTPA extractable Zn (mg/kg)	1.80	Medium	Landon (1991)
DTPA extractable Mn (mg/kg)	19.04	High	Landon (1991)
DTPA extractable Fe (mg/kg)	69.72	Very high	Landon (1991)
Sand (%)	40		
Silt (%)	7		
Clay (%)	53		
Textural class	Clay		Gee and Bauder (1986)

Table 2. Selected properties of Minjingu phosphate rock (MPR) used in the study.

Property	Average value
pH (H_2O)	8.50
Total P (%)	13.0
Bray I P (%)	0.01
Solubility in NAC (%)	3.60
Ca (%)	35.5
$CaCO_3$ (%)	6.90
K_2O (%)	1.40
MgO (%)	3.40

Table 3. Selected properties of composts used in the study.

Compost type	Moisture	OC	Total N	Total P	Total K	Total Ca	C:N	pH_w
	%							
CP†	26.42	46.91	1.61	0.15	2.87	4.38	29.1	8.26
CPMPR††	29.08	38.3	1.43	1.39	2.28	7.37	26.8	8.54

†, CP = Coffee pulp composted alone; ††, CPMPR= Coffee pulp composted with MPR.

Effect of P source and application rate on P uptake

Generally, P uptake increased with increasing rates of CP, MPR and CPMPR (Figure 1). The differences in P uptake between plants treated with CP, MPR and CPMPR was only significant ($p = 0.05$) at application rate rates above 40 mg P/kg soil due to high P fixing capacity of the soil. The overall mean plant P uptake values observed for the plants treated with CP, MPR and CPMPR at 20 to 100 mg P kg^{-1} soil were 11, 13 and 18 times P uptake of control plants which did not receive external P.

The least effect of CP on P uptake as compared to MPR and CPMPR (Figure 1) was due to low amounts of

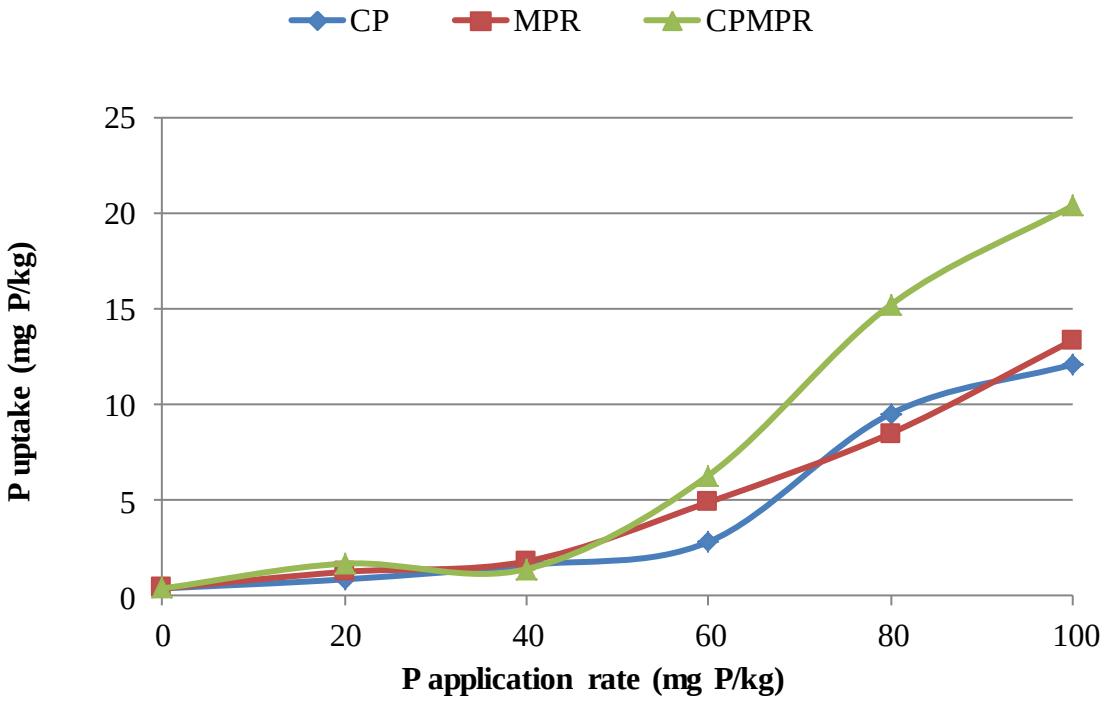

Figure 1. The effect of P sources and application rates on P uptake.

plant available P in the coffee pulp composted alone when compared to plant available P in MPR and CPMPR. Observed increase in P uptake from CP could be attributed to P released after mineralization of the coffee pulp and competition of the humic substances for P fixation sites of the soil, thus increasing P availability for plant uptake. Decomposition products are also reported to have effect on soil structure and moisture retention which enhances microbial activities and ultimately soil P turn over (Ikerra et al. 2006; Olumuyiwatogun et al., 2004). Although MPR increased P uptake; the increase was less than what was observed following application of CPMPR due to slow and continuous dissolution of singly applied MPR as transformation of some P released from MPR into plant unavailable forms.

The highest increase in P uptake observed for CPMPR treated plants was attributed to increased MPR dissolution rate and hence P availability as influenced by the effect of low molecular weight organic acids and humic substances released during microbial decomposition of coffee pulp. Extra P released in plant available from upon decomposition and mineralization of the coffee pulp and reduction of P fixation sites on the soil colloidal surfaces also contributed on increased P uptake. Based on the observed variations in P uptake among CP, MPR and CPMPR treated plants, it was worth to conclude that composting coffee pulp with MPR increased the availability of P for plant uptake as compared to singly composted coffee pulp and MPR applied alone.

Effect of P source and application rates on plant tissue P contents

Application of 20 to 100 mg P kg-1 in the form of CP, MPR and CPMPR had positive and significant effect on plant P concentration (Figure 2). The general effect of P source on plant P concentration was in the order CP < MPR < CPMPR. The increase in P concentration in CP, MPR and CPMPR treated plants were 23%, 36% and 110%, respectively above the control. Observed increase in plant tissue P concentration further implied that, composting coffee pulp with Minjingu phosphate rock increased the dissolution of MPR hence the release of P in plant available form for plant uptake uptake.

Ditta et al. (2017) reported higher P accumulation in chick pea grains and straws following application of PR enriched composts as compared to P content of similar plant parts from plants receiving non enriched composts. Research findings by Dzung et al. (2013) reported improvement in soil fertility , mineral nutrients in the coffee leaf and growth rate of the coffee plant treated with composted coffee husk as compared with plants on plots which didn't receive the compost. Meena and Biswas (2015) reported 68.8 to 58.7% higher residue Olsen-P in in soils treated with phosphate rock enriched composts over control plots. These research findings are therefore

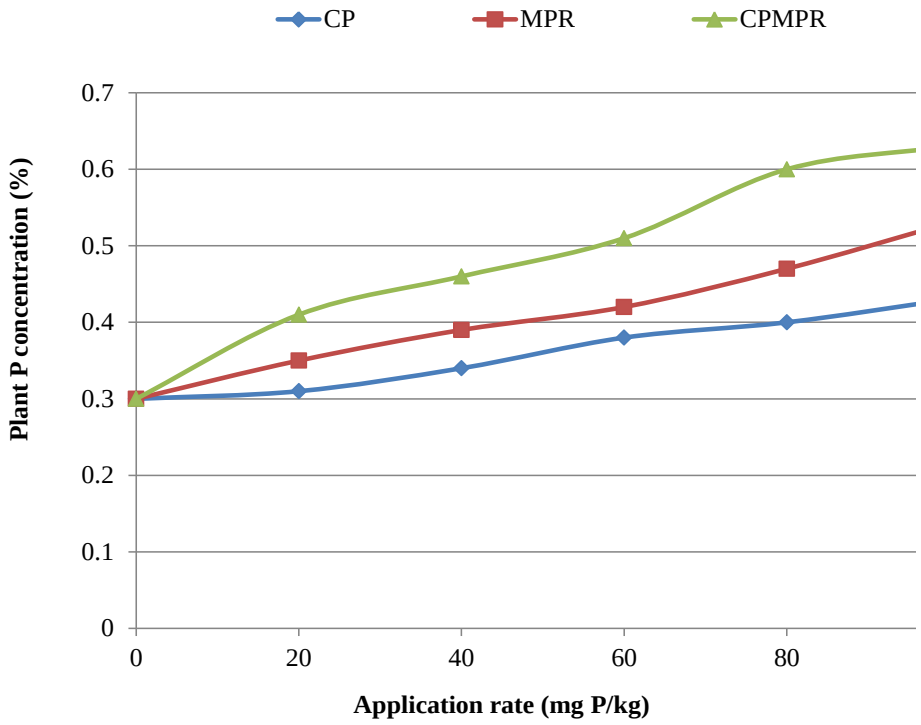

Figure 2. Effect of P sources and application rates on tomato plant P concentration.

in agreement with our findings and support the conclusion that observed higher P uptake and P accumulation in plants treated with CPMPR as compared to plants treated with CP or MPR was due to improved PR dissolution and therefore P availability for plant uptake.

Conclusion

From this study, increased P uptake was observed due to application of coffee pulp compost, Minjingu phosphate rock and coffee pulp composted with Minjingu phosphate rock as source of plant available P. The observed increase in P uptake and its concentration in plant tissue due to CP, MPR and CPMPR application indicate an increased availability of phosphorus for plant uptake. Despite the application of all materials at the same rates of phosphorus, there existed a variation in plant P uptake between different P sources indicative of the differences in P availability. The highest and significant effect observed with CPMPR indicates that composting coffee pulp with MPR is a potential technique for improving the availability of P from MPR and subsequent tomato P uptake.

CONFLICT OF INTERESTS

The authors declare that they have no conflict of interest.

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REFERENCES

Berecha G, Lemessa F, Wakjira M (2011). Exploring the Suitability of Coffee Pulp Compost as Growth Media Substitute in Greenhouse Production. International Journal of Agricultural Research 6:255-267.

Bremner JM (1996). Nitrogen Total. In: Methods of Soil Analysis part 3. Chemical Methods. SSSA Book series No.5. pp. 1085-1121.

Buehler S, Oberseson A, Rao IM, Friesen DK, Frosaad E (2002). Sequential phosphorus extraction of a P-labeled oxisol under contrasting agricultural system. Soil Science Society of America Journal 66:868-877.

Buresh JR, Smithson PC, Hellmus DT (1997). Building soil phosphorus capital in Africa. In: Proceedings of an International Symposium held at the 88th Annual meeting of the American Society of Agronomy and Soil Science Society of America. (Edited by Buresh, J. R., Sanchez, P. A. and Calheun, F.), 6 November, 1996, Indiana, USA. 111-149pp.

Cubero-Abarca R, Moya R, Valaret J, Filho MT (2014). Use of coffee (Coffea arabica) pulp for the production of briquettes and pellets for heat generation. Ciência e Agrotecnologia Lavras 38(5):461-470

Dierolf TS, Fairhust TH, Mutert EW (2001). Soil Fertility Kit: A Toolkit for

Acid Upland Soil Fertility Management in South east Asia. Potash and Phosphate Institute, Canada 149 p.
Ditta A, Muhammad J, Imtiaz M, Mehmood S, Qian Z, Tu S (2017). Application of rock phosphate enriched composts increases nodulation, growth and yield of chickpea. International Journal of Recycling of Organic Waste in Agriculture 7:33-40.
Dzung NA, Dzung TT, Khanh V (2013). Evaluation of Coffee Husk Compost for Improving Soil Fertility and Sustainable Coffee Production in Rural Central Highland of Vietnam. Resources and Environment 3(4):77-82
Food and Agriculture Organization (FAO) (1987). Soil Management: Compost production and use in tropical and subtropical environments. FAO Soils Bulletin Rome 56:177.
Food and Agriculture Organization (FAO) (2000). Fertilizers and their use: A pocket guide for extension officers. 4th Ed. Food and Agriculture Organization of the United Nations, International Fertilizer Industry Association, Rome 70 p.
Gee GW, Bauder JM (1986). Particle size analysis. In: Methods of soil analysis Part 1, Physical and mineralogical Methods. (Edited by Klute, A.), SSSA Inc. Madison, Wisconsin, United States of America pp. 383-411.
Graves ER, Hattemer GM (2000). Composting. In: Environmental Engineering Part 637. National Engineering Handbook. USDA. 88pp.
Ikerra ST, Semu E, Mrema JP (2006). Combining Tithonia diversifolia and Minjingu phosphate rock for improvement of P availability and maize grain yields on a chromic acrisol in Morogoro, Tanzania. Nutrient Cycling in Agro Ecosystems 76:249-260.
Kpomblekou AK, Tabatabai MA (2003). Effect of low-molecular weight organic acids on phosphorus release and phytoavailabilty of phosphorus in phosphate rocks when added to soils. Agriculture, Ecosystems and Environment 100:275-284.
Kuo S (1996). Phosphorus. In: Methods of Soil Analysis Part 3. Chemical Methods. SSSA Book Series 5:475-490.
Landon JR (1991). Booker Tropical Soil Mannual. A handbook for soil survey and agricultural land evaluation in the tropics and subtropics. Paperback Edition. Addison Wesley Longman Limited, England 474 p.
Lindasy WO, Norvell WA (1978). Development of a DTPA soil test for zinc, iron, manganese and copper. Soil Science Society of America Journal 42:421-428.
Mclean EO (1982). Soil pH and lime requirement. In: Methods of Soil Analysis Part 2. Edited by Page, A.L., Miller, R.H. and Keeny, D.R., American Society of Agronomy. Madson, Wisconsin pp. 192-223.
Meena MD, Biswas DR (2015). Effect of rock phosphate enriched compost and chemical fertilizers on microbial biomass phosphorus and phosphorus fractions. African Journal of Microbiology Research 9(23):1519-1526
Mihreteaba HT, Cegliea FG, Alya A, Tittarellib F (2015). Rock phosphate enriched compost as a growth media component for organic tomato (*Solanum lycopersicum* L.) seedlings production. Biological Agriculture and Horticulture. DOI: 10.1080/01448765.2015.1016114
Mowo JG, Janssen BH, Oenema O, German LA, Mrema JP, Shemdoe RS (2006). Soil fertility evaluation and management by smallholder farmer communities in the Northern Tanzania. Agriculture, Ecosystems and Environment 116(1-2):47-59.
Nelson DW, Sommers LE (1992). Total organic carbon and organic matter. In: Methods of Soil Analysis Part 2. Edited by Page, A. L., Miller, R. H. and Keeny, D. R., American Society of Agronomy. Madson, Wisconsin pp. 421-428.
Núñez AC, Rentería IDCD, Villagómez MÁL, Martínez PE, Sánchez CEM, Pulido SAA, Rojas-Ronquillo R (2015). The use of coffee pulp as a potential alternative supplement in ruminant diets. Journal of Agricultural Science and Technology A5:214-217.
Okalebo JR, Gathua KW, Woommer PL (1993). Laboratory methods of soil and plant analysis. A working manual. KARI and Rost 88 p.
Olumuyiwatogun A, Babatunde AW, Adediran JA (2004). Growth, nutrient uptake and yield of tomato in response to different plant residue compost. Available at: https://www.researchgate.net/publication/267993824_Growth_nutrient_uptake_and_yield_of_tomato_in_response_to_different_plant_residue_compost
Pandeya A, Soccol CR, Nigam P, Brand D, Mohan R, Roussos S (2000). Biotechnological potential of coffee pulp and coffee husk for bioprocesses. Biochemical Engineering Journal 6:153-162.
Preethu DC, Bhanu Prakash BNUH, Srinivasamurthy CA, Vasanthi BG (2007). Maturity indices as an index to evaluate the Quality of Compost of coffee waste Blended with Other Organic wastes. In: Proceedings of the International Conference on Sustainable Solid Waste Management. 5-7 September 2007, Chennai, India pp. 270-275.

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Full Length Research Paper

Association mapping for flag leaf thickness in an *indica* rice population from South China

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Flag leaf is the most important source of photosynthate for developing rice grains, and flag leaf thickness is an important morphological trait in rice plant-type breeding programs. In the present study, we carried out association mapping for flag leaf thickness in a local rice population which consisted of 86 cultivars derived from breeding programs and planted in large areas in South China. Phenotyping was conducted in the field using nondestructive leaf thickness measurements. Two hundred and thirty-six SSR markers covering 12 chromosomes were employed to genotype the accessions. The association analysis was carried out using a unified mixed-model approach. The Q+K model was selected for investigating marker-trait associations. A total of eleven marker-trait pairs with significant marker-trait associations were identified which were distributed on eight chromosomes. Four of these loci had already been identified as related to flag leaf thickness in previous studies, while the other seven were novel QTLs. The locus PSM163 had the highest r^2-marker value of the seven novel loci, explaining 21.54 and 18.49% of the phenotypic variation in 2008 and 2009, respectively. Three of four QTLs, which were detected in a F_2 mapping population in the validation study, could correspond to a significant locus in AM, respectively. The six alleles which had the highest phenotypic values at their respective loci should be considered as favored alleles in breeding programs. Pyramiding the favored alleles for flag leaf thickness identified in the study might be a valuable approach to construct an ideal plant architecture in rice breeding.

Key words: Rice, flag leaf thickness, breeding programs, association mapping, mixed linear model.

INTRODUCTION

Rice is a staple food for about 50% of the global population. With the ongoing reduction in arable landcaused by urbanization and industrialization, breeding rice varieties with greater yield potential will be a very important component of meeting the increased food demand of a growing global population. Genetic improvement of plant morphology is the backbone of increasing rice yielding potential (Khush, 1995; Yuan, 1997). Flag leaf is the most important source of photosynthate for developing rice grains. Over 50% of the carbohydrate accumulated in rice grains is produced by flag leaves (Gladun and Karpov, 1993). Flag leaf

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morphology is a major determinant of plant architecture, canopy photosynthesis and grain yield potential in rice (Jin et al., 2018). Rice leaf thickness is significantly negatively correlated with Ls (stomatal limitation under high light) and Ls_{low} (stomatal limitation under low light) but significantly positively correlated with Fv/Fm (maximum PSII efficiency), showing that thick leaves are beneficial in increasing the photosynthetic rate and carbohydrate assimilation (Qu et al., 2017). Thick leaves are favorable for improving P_n and canopy photosynthesis in rice, so leaf thickness has been regarded as one of the major indices in rice cultivation and plant-type breeding programs (Peng, 2000; Zhu et al., 2016). In recent decades, several plant-type models proposed by cultivators and breeders have employed thick flag leaves as a main selection index in rice cultivation and breeding programs. Matsushima (1976) suggested that the flag leaf should be “short, thick, erect” for the rice ideotype. Similarly, thick flag leaves were proposed in other ideotype models in rice breeding (Yang et al., 1984; Kush, 1995; Yuan, 1997).

The relationship between leaf thickness and grain yield and yield traits in *indica* rice had been analyzed by Chen et al. (2011) and Liu et al. (2014). The studies revealed a tight correlation between leaf thickness and panicle traits. In the studies, the thickness of the top three leaves had a significant negative correlation with leaf angle and the number of panicles per plant and a significant positive correlation with leaf length, panicle length, number of primary branches, number of secondary branches, filled grains per panicle, grain density, grain weight per panicle, and number of spikelets per panicle. There was little correlation with seed setting rate, 1000-grain weight, and harvest index. Thicker leaves were not just favourable to larger panicles and higher grains weight per panicle, but also to the construction of an ideal plant architecture in rice (Liu et al., 2014). The results also showed that flag leaf thickness is closely related to the thickness of other leaves on the same stem, suggesting that rice leaf thickness traits share a single genetic system controlled by multiple genes or quantitative trait loci (QTL) (Liu et al., 2014).

In recent years, many genes or QTLs related to the flag leaf morphological traits in rice, such as flag leaf length (Jiang et al., 2010; Shen et al., 2012), flag leaf width (Fujino et al., 2008; Qi et al., 2008), flag leaf area (Wang et al., 2011), flag leaf angle (Sakamoto et al., 2006), and rolling leaf (Zhou et al., 2010), have been cloned or fine-mapped. However, so far no QTLs for flag leaf thickness have been identified based on phenotypic data directly measured in the field, although a few QTLs for SLW or SLA of flag leaf had been identified in rice (Laza et al., 2006; Kanbe et al., 2008; Zhao et al., 2008; Takai et al., 2010).

Association mapping (AM) is a high-resolution method for the identification of QTLs for complex genetic traits in plants (Mackay and Powell, 2007). It has at least three benefits compared with traditional linkage analysis: consuming far less time, a higher mapping resolution, and a greater allele number (Breseghello and Sorrells, 2006a; Zhu et al., 2008). Association mapping has recently been successfully used to identify marker-trait associations in various plant species, such as maize (Pace et al., 2015), *Arabidopsis thaliana* (Davila Olivas et al., 2017), barley (Kraakman et al., 2006; Wang et al., 2017), wheat (Sabiel et al., 2017), and soybean (Che et al., 2017). In rice, Agrama et al. (2007) used a mixed linear model method to detect marker-trait associations for yield and its components in 103 accessions genotyped using 123 SSR markers. Twenty-five associations were identified. Zhao et al. (2013) genotyped 130 rice accessions using 170 SSR markers to identify marker–trait associations for physicochemical traits affecting eating quality. In total, 101 marker–trait associations (p<0.05) were identified with 52 different SSR markers covering 12 chromosomes. Fujino et al. (2015) used 115 SSRs for genotyping in an association analysis of 63 cultivars derived from rice breeding programs in Hokkaido, Japan. Six QTLs were identified for heading date and seventeen for low temperature germinability. Dong et al. (2018) newly found four loci associated with flag leaf inclination in rice by association mapping. Today, association analysis has become a powerful method of gene digging for complex traits in rice (Zhai et al., 2018; Huang and Han, 2018).

South China is one of the major rice-producing and consuming regions in China, which expands the area between the latitude of 18°43ǫ and 26°24ǫ N and the longitude of 104°26ǫ and 117°19ǫ E including Guangdong, Guangxi, and Hainan provinces (Figure 1). In this region, the tropical and subtropical monsoon climate is typical, and the temperature and precipitation resources are rich (more than 300 d with the daily mean temperature of 10°C and annual rainfall of 1400–2000 mm). Indica rice is traditionally planted in a double-cropping system in South China, and the rice area accounted for 15.1% of the total national rice acreage (Liu and Zhang, 2010).

In the present study, an association analysis was carried out between flag leaf thickness and SSR markers employing a set of elite rice cultivars derived from breeding programs in South China during the past 60 years. The objective of this study was to identify major QTL(s) associated with flag leaf thickness which could help us to detect the genetic mechanism of flag leaf thickness in rice and be used in rice molecular breeding to construct an ideal plant type.

MATERIALS AND METHODS

Plant material and phenotyping

Eighty-six semidwarf *indica* rice cultivars were used as the association panel in this study, which included 80 cultivars

Figure 1. Map of the area of South China in which double-cropping rice are cultivated. *The marked black area in the map is the area of South China.

developed in South China from 1949 to 2006, four cultivars introduced from IRRI, and two landraces which were used as core parents in rice breeding programs in South China (Table 1). Seeds of the cultivars were preserved in the rice germplasm repository of the Rice Research Institute, Guangdong Academy of Agricultural Sciences.

Field experiments were carried out at the test station of the Rice Research Institute, Guangdong Academy of Agricultural Sciences, Guangzhou, in the late-cropping seasons in 2008-2009. The field trials design and field management were conducted according to the methods described by Liu et al. (2014). Five to seven days after full heading, 10 plants with uniform growth were sampled from each plot to determine the thickness of the flag leaf blade on the main stem. The measurement the protocol as described by Liu et al. (2014) was used for measuring the thickness of the flag leaf. To avoid interference from leaf water status, the field was kept flooded while measuring of flag leaf thickness.

SSR genotyping

Two hundred and thirty-six polymorphic SSR markers were employed to genotype the cultivars. The average distance between loci was about 6.4 cM. The number of markers on chromosomes 1 to 12 was 27, 24, 25, 21, 16, 19, 18, 21, 16, 14, 18 and 17, respectively (Figure 2). One hundred and fifty-two SSR markers were obtained from the Gramene database (http://www.gramene.org), while the remaining SSR markers (labeled ‘‘PSM’’) were developed in the State Key Laboratory for Conservation and Utilization of Subtropical Agro-Bioresources based on the sequence of the delimited region from the International Rice Genome Sequencing Project (IRGSP) database (http://rgp.dna.affrc.go.jp/IRGSP/index.html). Mini-scale DNA was extracted using the modified SDS protocol reported in Zheng et al. (1995). PCRs were conducted according to the method described by Panaud et al. (1996) in a 20 µl reaction mix containing 50 ng

Table 1. Rice cultivars included in this study and their origin.

S/N	Cultivar name	Released or introduced year	Subpopulation*
1	CYZ-18	1974	4
2	QIU-EA	1970	2
3	GYA-C17	1976	5
4	GQA	1963	2
5	GNA-1	1969	2
6	QBZ-3	1970	2
7	YXYZ	2005	2
8	FAZ	1999	1
9	TSA-2	1992	3
10	YQL	1982	2
11	WHA-1	1983	2
12	YXZ	1998	1
13	ZGA-1	1990	3
14	CG-314	1980	2
15	ZERZ	2001	2
16	ST-1	1999	1
17	ZYQ-8	1973	6
18	LQZ-1	1988	1
19	CYZ-18-X	1974	4
20	AZZ-4		4
21	QDZ	1992	1
22	PG-2	1971	4
23	RPA	1964	2
24	QFA	1971	7
25	GLA-4	1969	4
26	XZ-69	1973	6
27	FBZ	2001	1
28	GC-2	1976	2
29	MBYZ	2001	3
30	FAZ-5	1998	5
31	TXZ-25	1998	6
32	GE-104	1976	3
33	GEA-5	1963	7
34	GYA-121	1976	2
35	AXZ	2003	1
36	SZZ	1966	6
37	JEA	1967	7
38	MXZ	1968	7
39	HMZ	1967	7
40	MLSM	2005	1
41	FMZ	2005	7
42	GJ-9	1964	4
43	GC-13	1977	2
44	ZZA-11	1962	4
45	JX-89	1991	6

Table 1. Contd.

46	YFZ	2001	3
47	XXZ	1995	5
48	HHZ	2005	3
49	GNZ	2005	7
50	QLSM	2004	7
51	IR24	1971	6
52	QLA	1990	5
53	IR22	1971	6
54	IR8	1971	6
55	SG-36	1986	5
56	FQA	1992	7
57	SG-1	1982	5
58	XX-299	1992	7
59	QGZ-25	1985	5
60	QJZ	1986	5
61	YXZ-8	2005	6
62	QIN-EA	1975	7
63	FHZ	2002	7
64	SC-169	1983	7
65	TXZ-13	1996	6
66	MLXZ	2001	5
67	QXZ-3	1992	6
68	SEA	1983	7
69	YEZ	2005	3
70	JDL		4
71	AJNT	1957	4
72	GSA	1984	7
73	TQ-2	1984	7
74	YG-146	1988	5
75	QSZ	1991	7
76	FAZ-1	1997	7
77	QXJZ	1960	4
78	SYA	1992	7
79	GCA-3784	1959	4
80	JLXSZ	1982	6
81	GES	1979	5
82	SY-2	1994	7
83	GCA-4182	1959	4
84	LHZ	1996	7
85	IR20	1971	6
86	AQZ	1995	7

template DNA, 200 µM dNTP, 1×PCR buffer, 1U *Taq* DNA polymerase, and 0.15 µM forward and reverse primers. DNA amplification was performed using a PTC-100™ 96 Plus thermal cycler. The reaction program was as follows: 94°C for 5 mins followed by 35 cycles at 94°C for 1 min, 55°C for 1 min, 72°C for 1 min with a final extension of 5 minutes at 72°C. PCR products were separated by size with 6% polyacrylamide gel electrophoresis and detected by silver staining.

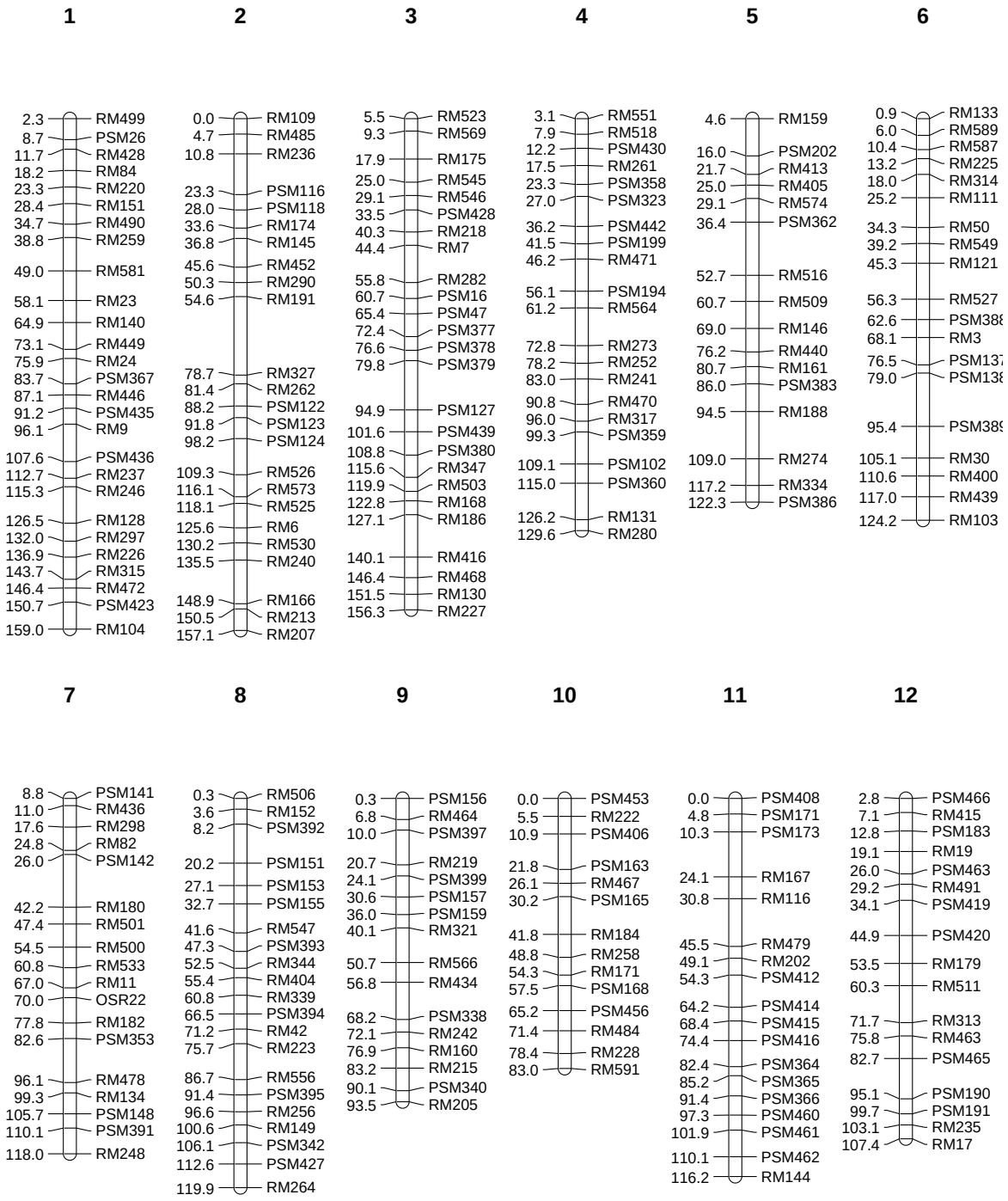

Figure 2. Distribution of 236 tested SSR markers on 12 rice chromosomes.

Genetic diversity and association analysis

Phenotypic data analysis

The data collection and basic processing was done in Excel 2007 on Windows XP. The distribution and histogram of flag leaf thickness were determined in SPSS 16.0 (SPSS Inc., Chicago, IL, USA) using the FREQUENCIES option in DESCRIPTIVE STATISTICS. Analysis of variance was carried out in SPSS 16.0 using the general linear model (GLM) and assuming a random effects model on multiple environments. Broad-sense heritability was calculated as Zhang et al. (2014).

Allelic diversity and demographic analysis

All cultivars were treated as pure lines. A few heterozygous loci were treated as missing data which were detected in this study. The

number of alleles and polymorphism information content (PIC) per locus were calculated with the Power Marker 3.25 program (Liu and Muse, 2005). A phylogenetic tree was constructed using the neighbor-joining method in MEGA 5.1 (Tamura et al., 2011). The population structure of the association mapping population was determined with the model-based STRUCTURE 2.3 program (Pritchard et al., 2000) using a burn-in of 10,000, a run length of 100,000, and a model allowing for admixture and correlated allele frequencies. Five runs were performed, and the number of sub-populations (K) was set from two to ten. The most likely number of sub-groups was estimated by LnP(D) in the STRUCTURE output and an ad hoc statistical ΔK following Evanno et al. (2005). Rare alleles, those with a frequency of less than 5% in the panel were treated as missing data for the structure analysis. Analysis of molecular variance (AMOVA) was performed with the program ARLEQIUN 3.11 (Excoffier et al., 2005). The F_{ST} value, which measures the degree of differentiation of each subpopulation, was calculated through AMOVA.

Association mapping

Association tests were performed with the mixed linear model (MLM) method in TASSEL 2.1 (http://www.maizegenetics.net). To reduce the type I error rate, four models, namely the Simple model, Q, K, and Q+K models, were used to evaluate the marker-trait associations. The population structure matrix (Q) was determined by running Structure 2.3 with the most likely number of sub-groups, K. The relative kinship matrix (K-matrix) was obtained using the software SPAGeDi (Hardy and Vekemans, 2002). Output from SPAGeDi was formatted to a text file readable by TASSEL 2.1. The best-fit model for the marker-trait association was determined using the Bayesian information criterion (BIC) to evaluate the four models: Simple model, Q, K, and Q+K. For controlling the type I error rate, *p*-values were compared to a Bonferroni threshold to identify significant loci (Nakagawa, 2004). The Bonferroni threshold was 1/236=0.0042, where 236 was the number of association tests for the trait in this study. The allelic effects at a marker locus were estimated using the Probability of Difference (PDIFF) procedure in SAS 9.1 (SAS institute Inc, Cary, NC, USA) on the least square means (LSMEANS) of the phenotype data.

Validation of QTLs identified through AM

To validate the significant loci for flag leaf thickness through association mapping, we constructed an F_2 population with the cross QSZ/P205. QSZ was a modern *indica* rice cultivar with flag leaf thickness of 255.3 cm selected from the association panel. P205 was a japonica variety with flag leaf thickness 382.5 cm we screened from the germplasms preserved in the rice germplasm repository of the Rice Research Institute, Guangdong Academy of Agricultural Sciences. 297 individuals were investigated for phenotypic and genotypic assay. Identification of flag leaf thickness and SSR genotyping performed using the method described above. QTL analysis was conducted using the approach of composite interval mapping in the computer package Windows QTL Cartographer version 2.5 (Wang et al., 2007).

RESULTS

Natural variation in flag leaf thickness

Flag leaf thickness of the 86 cultivars was measured in the field on the fifth to seventh day after full heading in the late-cropping seasons of 2008-2009. The flag leaf thickness values were normally distributed (Figure 3). The minimum, maximum, mean, and standard deviation (*SD*) were 251.9, 371.3, 309.4, and 24.4 μm in 2008 (Figure 3A) and 255.6, 372.5, 312.3, and 24.2 μm in 2009 (Figure 3B), respectively. The broad sense heritability (h^2) of flag leaf thickness was 88.6%.

Genetic diversity

We identified 781 polymorphic loci across the 86 accessions. The number of alleles per locus varied from two to twelve, with the average being 3.309. We detected 113 heterozygous loci, accounting for 0.557% of the total of 20,296 loci. The average Nei's genetic diversity index was 0.442, ranging from 0.042 to 0.839. The average PIC value was 0.415, ranging from 0.041 to 0.792.

Population structure

Analysis of the population structure was performed using the model-based program STRUCTURE 2.3. The model of ΔK value calculation was used to determine the most probable K. A sharp peak of ΔK at K=7 was observed (Figure 4A), indicating that the population could be optimally grouped with K=7. We therefore divided the population into seven subpopulations, S1 to S7. A graphical bar plot was then generated showing the posterior membership coefficients for each accession (Figure 4B). A neighbor-joining tree based on the genetic distance matrix was constructed using MEGA 5.1 (Figure 4C). It revealed genetic relationships that were relatively consistent with the STRUCTURE-based membership assignments of the cultivars. Most of the cultivars in the same subpopulation were classified in the same cluster. However, for a few cultivars, such as QFA, GQA and GEA-5, the classified cluster did not coincide with the corresponding subpopulation.

Estimation of relative kinship

The relative kinship matrix (K-matrix) was obtained using the software SPAGeDi. 42.05% of the pairwise relative kinship estimates were equal to zero, 32.04% were less than 0.05, 10.8% were between 0.05 and 0.1, 5.39% were between 0.1 and 0.15, 9.73% were between 0.15 and 0.5, and the remaining 0.014% of the estimates were >0.5 (Figure 5).

Population differences

The distribution of molecular genetic variation among and within the seven subpopulations was estimated by

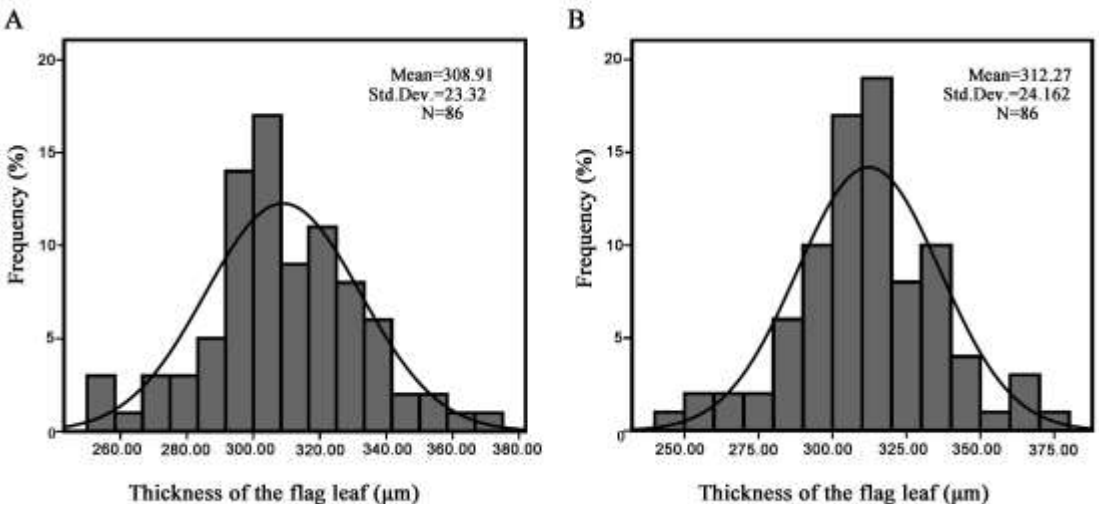

Figure 3. Histograms showing the frequency distribution of flag leaf thickness. (A) 2008; (B) 2009.

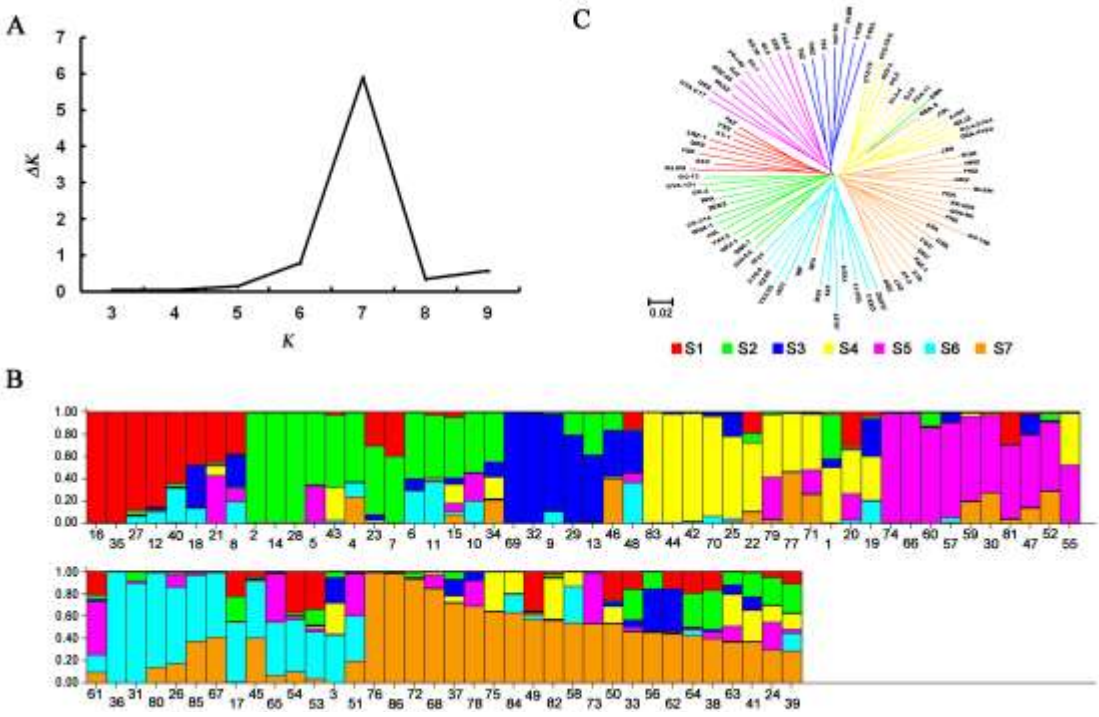

Figure 4. Population structure of 86 cultivars based on 236 SSR markers. (A) Changes of ΔK with the number of subpopulations; (B) Population structure analysis of 86 cultivars showing seven subpopulations (S1-S7), with the estimated membership probability listed on the y-axis and each cultivar represented by a thin vertical line in a different color; (C) Neighbor-joining tree analysis of the 86 rice accessions. The colors (S1-S7) correspond to the model-based populations.

AMOVA (Table 2). The variation among the subpopulations accounted for 7.18% of the total variation, whereas 92.82% of the variation was within the subpopulations. The pair-wise F_{ST} values between the seven subpopulations indicated that the levels of genetic divergence among subpopulations were medium to low (Table 3), on the whole. The highest was subpopulation S1 with S5 (0.105), and the lowest was S6 with S7 (0.025). The overall F_{ST} value was 0.072. It can be concluded that there is moderate genetic differentiation in

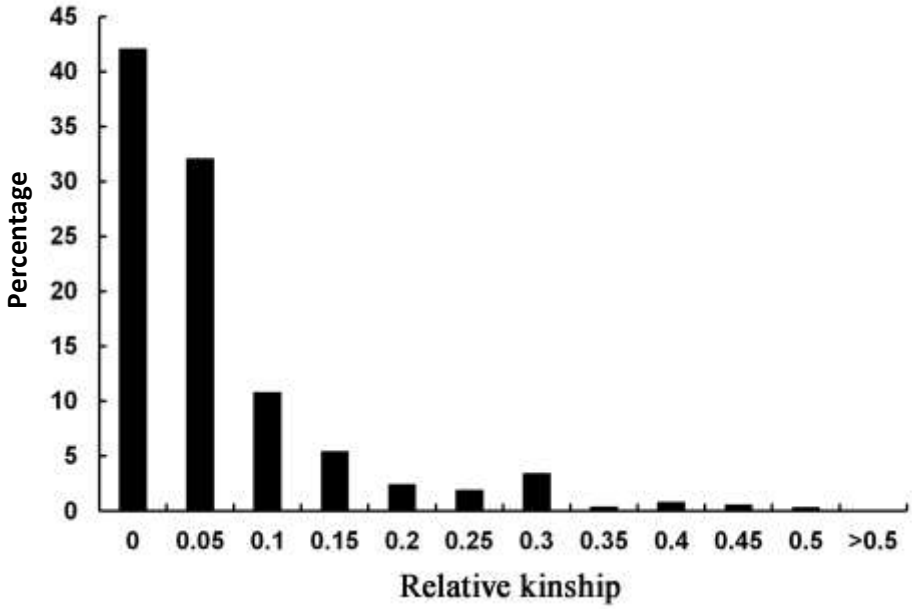

Figure 5. Distribution of pairwise relative kinship estimates among the 86 rice cultivars.

Table 2. AMOVA analysis of the seven rice accession clusters identified by STRUCTURE 2.3.

Source of variation	d.f.	Sum of squares	Mean squares	Estimated variance	Percentage variation (%)	*p*-value
Among clusters	6	114.73	19.12	0.71	7.18	<0.001
Among accessions within clusters	79	725.60	9.18	9.18	92.82	<0.001
Total	85	840.33	28.30	9.89		

Table 3. Pair-wise F_{st} values between the seven subpopulations as identified using Euclidean distance by the program STRUCTURE 3.1.

Cluster	S1	S2	S3	S4	S5	S6	Overall
S2	0.084	-					
S3	0.095	0.073	-				
S4	0.098	0.081	0.061	-			
S5	0.105	0.082	0.068	0.046	-		
S6	0.094	0.075	0.077	0.063	0.038	-	
S7	0.102	0.079	0.063	0.066	0.043	0.025	
Overall							0.072

the population.

Association mapping

To control for false positives (type I errors), four association mapping models (Simple model, Q, K, and Q + K models) were compared based on the Bayesian Information Criterion (BIC) and plots of observed-versus-expected *p*-values. Of the four models, the Q+K model had the smallest BIC values, the K model had the closest values to the Q+K model, and the Simple model had the largest BIC values (Table 4). We plotted the observed - versus-expected –log10(*p*) of the four models and found that the Q+K model had the smallest deviation between the observed and expected *p* values (Figure 6). The K model performed similarly to the Q+K model. This analysis indicates that the Q+K model is the best fit model for the correction of false positive in this association analysis, followed by the K model. The Q+K

Table 4. Fitness analysis of the mapping models for flag leaf thickness using Bayesian information criterion (BIC) among 86 cultivars genotyped with 236 molecular markers and phenotyped in Guangzhou, China in 2008-2009.

Model	2008	2009
Simple model	1675.56	1630.18
Q	1660.78	1635.40
K	1591.54	1572.70
Q+K	1566.76	1567.92

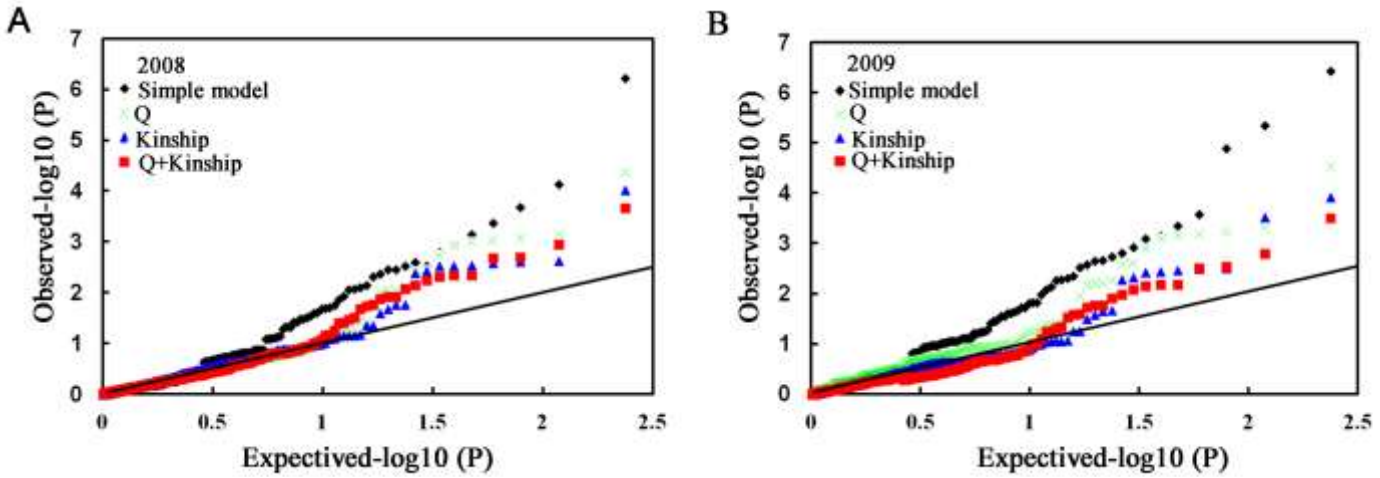

Figure 6. Quantile-quantile plots of estimated –log10(p) from association analysis using four models in flag leaf thickness. The black line is the expected line under the null distribution.

Table 5. Association of SSR markers with flag leaf thickness in *indica* rice.

Locus	Chr.	Position (cM)	2008		2009		Reference
			p-marker	r^2-marker	*p*-marker	r^2-marker	
RM297	1	132	-	-	0.0031	0.1214	
RM315	1	143.7	0.0019	0.1022	0.0009	0.1358	
PSM124	2	98.2	0.0031	0.1273	-	-	
RM227	3	156.3	0.0034	0.1136	0.0023	0.1406	Zhao et al. (2008); Khowaja and Price, 2008
RM471	4	46.2	-	-	0.0037	0.0736	
PSM362	5	36.4	-	-	0.0039	0.0928	Laza et al. (2006); Khowaja and Price, 2008
PSM353	7	82.6	0.0009	0.2507	0.0003	0.2261	Kanbe et al. (2008)
RM478	7	96.1	0.0019	0.0925	0.0008	0.1135	Zhao et al. (2008)
PSM163	10	21.8	0.001	0.2154	0.0006	0.1849	
PSM414	11	64.2	-	-	0.0035	0.0657	
PSM364	11	82.4	0.0024	0.1728	0.0037	0.1552	

model was therefore selected for the association mapping.

Association analysis was performed based on the Q+K model using the MLM procedure in TASSEL 2.1. A total of eleven marker-trait pairs were identified to have significant (p<0.0042) marker-trait associations (Table 5). These markers were distributed on eight chromosomes. On chromosomes 1, 7 and 11, two loci were identified. Only one locus was identified on chromosomes 2, 3, 4, 5 and 10. Four of these loci such as RM227 (Zhao et al., 2008; Khowaja and Price, 2008), PSM362 (Laza et al., 2006; Khowaja and Price, 2008), PSM353 (Kanbe et al., 2008) and RM478 (Zhao et al., 2008) had been already identified as related to flag leaf thickness. However the

Table 6. Comparison of the allelic effects of the six marker loci associated with flag leaf thickness.

Locus	Allele size (bp)[a]	Number of varieties	FLT (μm)[b]	
			2008	2009
RM315	134	33	297.3±22.6^{Cb}	303.6±26.5^{Bb}
	136	3	282.9±16.8^{Cb}	280.9±20.3^{Cc}
	139	32	338.6±26.7^{Aa}	341.6±29.2^{Aa}
	143	18	316.1±22.8^{Bb}	319.6±21.9^{Bb}
RM227	100	25	340.8±26.5^{Aa}	342.9±27.2^{Aa}
	106	41	305.6±31.6^{Bb}	308.6±32.3^{Bb}
	108	20	279.2±19.9^{Cc}	281.5±20.3^{Cc}
PSM353	293	49	288.5±23.5^{Bb}	291.6±22.7^{Bb}
	297	37	337.1±25.7^{Aa}	339.5±26.3^{Aa}
RM478	197	30	303.2±33.2^{Bb}	306.5±32.6^{Bb}
	199	26	338.2±25.7^{Aa}	339.8±26.7^{Aa}
	205	13	311.1±22.2^{Bb}	309.6±20.9^{Bb}
	208	17	275.7±19.1^{Cc}	282.1±16.4^{Bc}
PSM163	202	23	350.4±29.7^{Aa}	352.7±27.8^{Aa}
	206	37	310.2±21.1^{Bb}	312.3±22.5^{Bb}
	212	26	271.5±27.3^{Cc}	276.3±24.1^{Cc}
PSM364[c]	174	4	310.5±24.5^{Bb}	313.6±23.6^{Bb}
	178	29	294.7±18.2^{BbCc}	293.9±21.6^{BbCc}
	184	19	348.3±29.6^{Aa}	352.2±31.4^{Aa}
	186	25	332.9±22.7^{Aa}	337.6±34.8^{Aa}
	192	6	281.2±15.6^{Cc}	285.7±18.8^{Cc}

Note: [a] Allele Size (bp) is PCR product amplified by SSR markers; [b] Within a column, mean value±*SD* followed by capital and small letters represent significant difference at α=0.01 and 0.05, respectively; [c] Three heterozygotes identified with this marker were excluded from the data, 83 cultivars were used for the statistical analysis.

other seven loci were novel (Table 4). Six of the eleven loci were detected in both years, including RM315, RM227, PSM353, RM478, PSM163 and PSM364. The locus PSM353 (on chromosome 7) had the highest r^2-marker value in both years and explained 25.07 and 22.61% of the phenotypic variation respectively in 2008 and 2009. The second strongest association was with PSM163 (on chromosome 10), which explained 21.54 and 18.49% of the phenotypic variation in 2008 and 2009, respectively.

Allelic effects and their interactions

The allelic effects of the six loci were estimated which were detected in both years (Table 6). For RM315, individuals carrying the 139 bp allele (RM315^{139bp}) had thicker flag leaves than those carrying alleles RM315^{134bp} and RM315^{136bp}. Allele RM315^{139bp} thus had the highest positive impact on flag leaf thickness at this locus. For the other loci, the alleles RM227^{100bp}, PSM353^{297bp}, RM478^{199bp}, PSM163^{202bp} and PSM364^{184bp} had the highest phenotypic values and thus the greatest positive impact on flag leaf thickness. We therefore suggest considering RM315^{139bp}, RM227^{100bp}, PSM353^{297bp}, RM478^{199bp}, PSM163^{202bp} and PSM364^{184bp} favored alleles in breeding programs. The effect of the number of favor alleles on phenotypic values was also analyzed. As the number of favored alleles in an individual increased, so did the phenotypic value (Figure 7). The correlation between the number of favored alleles and flag leaf thickness was significantly positive, with a correlation coefficient of 0.726 (p<0.001). Cultivars with more favored alleles thus had greater flag leaf thickness.

Validation of significant loci through AM

To examine whether the QTLs identified through AM could be detected by bi-parental QTL mapping, we

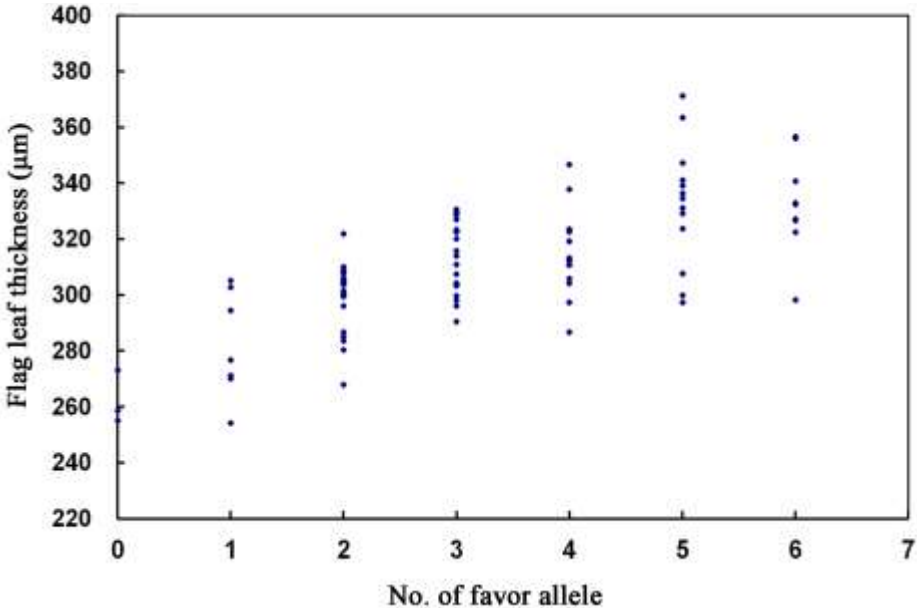

Figure 7. Relationship between the number of favored allele and flag leaf thickness in a cultivar.

Table 7. Summary of major QTLs for flag leaf thickness identified in the F_2 population of the cross QSZ/P205.

QTL	Chr.	Interval	LOD	Var (%)	Corresponding locus in AM
qFLT1.1	1	RM226-RM472	8.3	9.6	RM315
qFLT2.1	2	RM283-RM151	9.2	11.8	
qFLT7.1	7	RM505-RM18	12.1	15.7	PSM353
qFLT10.1	10	RM216-PSM165	9.6	10.5	PSM163

constructed one F_2 mapping population derived from the cross QSZ/P205. Four QTLs were detected in the study, which were located on chromosome 1, 2, 7 and 10, respectively (Table 7). In these loci, except qFLT2.1, all the other three QTLs could correspond to a significant locus in AM. qFLT1.1 explained 9.6% of the phenotypic variation, which was located in the interval between RM226 and RM472 on chromosome 1. The area of qFLT1.1 on chromosome overlaped the significant locus RM315 identified in AM. The QTL qFLT4.1 explained 15.7% of the phenotypic variation, which was located in the interval between PSM432 and RM18 on chromosome 4. The QTL qFLT4.1 was very near to the significant locus PSM353 detected in AM. The QTL qFLT10.1 explained 10.5% of the phenotypic variation, which was located in the interval between RM216 and PSM165 on chromosome 10 and overlaped the significant locus PSM163 in AM.

DISCUSSION

Flag leaf thickness is so important for high yielding in rice that breeders employed it as one of the major selecting targets in rice breeding programs (Yang et al., 1984; Krush, 1995; Yuan, 1997). In recent years, a few QTLs for flag leaf SLW and SLA, the alternative indicators of leaf thickness, have been identified in rice (Laza et al., 2006; Kanbe et al., 2008; Zhao et al., 2008; Takai et al., 2010). However, SLW and SLA are not direct phenotypic measurements, but the indexes converted from measurements of several traits. It is therefore difficult to ensure accuracy when these converted data are used as phenotypic data to identify genes (QTLs) for leaf thickness in rice. This may be the main reason why although several QTLs for SLW and SLA have been identified, no major QTL has been cloned or fine-mapped. In present study, we first carried out an association analysis between SSR markers and flag leaf thickness using the phenotypic data obtained with a nondestructive rice leaf thickness instrument and identified several QTLs for flag leaf thickness in *indica* rice.

Elite breeding materials in plant breeding programs could be used as a population for association analysis (Breseghello and Sorrells, 2006b; Zhu et al., 2008).

Genetic improvement programs of plant cultivars could be considered recurrent selection breeding programs in the breeding history of a local region. In each round of selection, genes introgressed from the primary groups or exotic germplasm caused phenotypic changes. Genomes in local populations have been structured by artificial selection of genotype × environment interactions during plant breeding programs (Shinada et al*.,* 2014). This offers two advantages for association mapping focused on elite lines derived from breeding programs in a local region: first, precise evaluation of phenotypes can be accomplished because the elite lines are genetically stable and well adapted to the local environmental conditions; second, elite lines are often used as parents for crossing in the next round of breeding, and significant markers associated with target traits could thus be used for marker-assisted selection in the progenies (Breseghello and Sorrells, 2006b; Zhu et al., 2008). Several association studies employing a local population derived from crop breeding programs have been conducted. Breseghello and Sorrells (2006a) performed association mapping for kernel size and milling quality using an association panel consisting of 95 elite soft winter wheat cultivars which were genotyped using 36 SSRs. The selected cultivars represented the variability of the current elite soft winter wheat cultivars in the eastern United States. The analysis identified 62 significant marker-trait association loci. Fujino et al*.* (2015) used 115 SSRs for genotyping in an association analysis of 63 cultivars derived from rice breeding programs in Hokkaido, Japan. Six QTLs were identified for heading date and seventeen for low temperature germinability. An association panel consisting of 109 German winter barley cultivars which were released in Germany between 1959 and 2003 was genotyped using 72 SSRs to detect loci related to grain yield traits, and 91 significant marker-trait loci were identified (Rode et al*.,* 2012). Three hundred and sixty-three elite breeding lines were selected for genotyping from an IRRI irrigated rice breeding program and genotyped for 71,710 SNPs using genotyping-by-sequencing (GBS), and 52 QTL for 11 agronomic traits were identified through association analysis (Begum et al., 2015). In the present study, an association panel consisting of 86 cultivars which had once been planted in large areas in South China was selected as a panel for association mapping of flag leaf thickness in rice. These cultivars, which were selected from 334 modern cultivars and some landraces in South China, included two landraces, four foreign germplasms from IRRI and 80 improved cultivars released in 1957-2005. This population was representative of the variability of the elite *indica* rice germplasm released in the South China since 1949. Two hundred and thirty-six SSR markers covering 12 chromosomes were employed for genotyping the accessions. The population size was similar to that in studies by Breseghello and Sorrells (2006a), Fujino et al*.* (2015), and Rode et al *.* (2012) (95, 63 and 109 varieties, respectively). However, the SSR density was significantly higher than that of the aforementioned three studies (which used 36, 115 and 72 SSRs, respectively). We identified 781 polymorphic alleles, and the mean number of alleles per locus was 3.309, with a range from 2 to 12. This was similar to the 3.88 alleles reported in a rice core collection (Zhang et al*.,* 2011) and the 3.9 alleles in an association mapping population identified by Jin et al*.* (2010). The average PIC value was 0.415, ranging from 0.041 to 0.792. This is similar to that reported by Jin et al*.* (2010) in a rice panel (0.4214), but slightly higher than the result determined by Cui et al*.* (2013) in a diverse rice panel (0.3137) and Xu et al. (2016) in rice collected from China (0.2465). The average Nei's genetic diversity index was 0.442, ranging from 0.042 to 0.839. This is similar to the 0.3413 reported by Cui et al*.* (2013) and the results reported by Yu et al. (2013).

Spurious associations between candidate markers and phenotypes can be caused by population structure, relatedness between individuals, selection, or genetic drift (Yu and Buckler, 2006). A unified mixed-model approach which demonstrated improved control over other methods for both type I and type II error rates was introduced by Yu et al*.* (2006). It was successfully used in association mapping (Breseghello and Sorrells, 2006a; Agrama et al., 2007; Yang et al*.,* 2010; Neumann et al*.,* 2011). In the present study, we compared four models, the Simple, Q, K, and Q+K models. The Q+K model had the smallest BIC value and best approximated the expected cumulative distribution of *p* values. This indicated that the Q+K model was the optimal model for the identification of marker-trait loci in a local population derived from breeding programs.

We identified 11 marker-trait association loci (Table 5). Seven of these were novel QTLs identified in this study, while four had been previously reported. The locus RM227 on chromosome 3 was detected in both the 2008 and 2009 data, and it has been reported as associated with both SLW (Zhao et al*.,* 2008) and SLA (Khowaja and Price, 2008). The locus PSM362 on chromosome 5 was detected in the 2009 data and has been identified as linked to SLA by Laza et al*.* (2006) and Khowaja and Price (2008). The locus PSM353 on chromosome 7 had the highest r^2-marker value in both years (0.2507 and 0.2261); it is located in the region of a reported QTL associated with SLW (Kanbe et al*.,* 2008). The locus RM478 was detected in both the 2008 and 2009 data and has been reported to affect SLW by Zhao et al. (2008). Of the seven novel loci, the locus PSM163 had the highest r^2-marker value, explaining 21.54% and 18.49% of the phenotypic variation in 2008 and 2009, respectively.

In the validation study, three of four QTLs detected by one F_2 mapping population could correspond to a significant locus in AM. It demonstrated that almost all of the significant loci identified in AM could be detected in bi-parental F_2 groups. The results also indicated that AM

is more efficient and has a greater allele number than bi-parental QTL mapping. Six alleles, RM315^{139bp}, RM227^{100bp}, PSM353^{297bp}, RM478^{199bp}, PSM163^{202bp} and PSM364^{184bp}, had the highest effect at their respective loci (Table 6). They should therefore be considered as favored alleles in breeding programs. The significantly positive correlation between the number of favored alleles and flag leaf thickness shows that pyramiding several of the favored alleles is a viable approach to improve flag leaf thickness and construct an ideal plant type in rice breeding programs.

Conclusion

Association mapping is an important approach for identifying QTLs based on linkage disequilibrium. In the present study, a total of eleven marker-trait pairs with significant marker-trait associations were identified. The putative QTLs were distributed on eight chromosomes. Seven loci were novel QTLs. Almost all of the significant loci identified in AM could be detected in bi-parental F_2 groups and indicated that AM is more efficiency and has a greater allele number than bi-parental QTL mapping. Pyramiding the favored alleles for flag leaf thickness identified in this study will be a valuable approach to construct ideal plant architecture in rice ideal plant type breeding programs.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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REFERENCES

Agrama HA, Eizenga GC, Yan W (2007). Association mapping of yield and its components in rice cultivars. Molecular Breeding 19:341-356.

Begum H, Spindel JE, Lalusin A, Borromeo T, Gregorio G, Hernandez J, Virk P, Collard B, McCouch S R (2015). Genome-wide association mapping for yield and other agronomic traits in an elite breeding population of tropical rice (Oryza sativa). PLoS One 10:e0119873.

Breseghello F, Sorrells ME (2006a). Association mapping of kernel size and milling quality in wheat (*Triticum aestivum* L.) cultivars. Genetics 172:1165-1177.

Breseghello F, Sorrells ME (2006b). Association analysis as a strategy for improvement of quantitative traits in plants. Crop Science 46:1323-1330.

Che Z, Liu H, Yi F, Cheng H, Yang Y, Wang L, Du J, Zhan P, Wang J, Yu D (2017). Genome-wide association study reveals novel loci for SC7 resistance in a soybean mutant panel. Frontiers in Plant Science 8:1771.

Chen YD, Zhou XQ, Chen DG, Li LJ, Li JC, Zhang X (2011). Studies on the differences of morphological characteristics for different high-yielding rice types in the late crop environmental condition. In: Chen Y D and Zhang X eds, *Researches on dynamic plant type in indica rice*. Shanghai Scientific & Technical Publishers, Shanghai, China, pp. 28-38.

Cui D, Xu C, Tang C, Yang C, Yu T, A X, Cao G, Xu F, Zhang J, Han L (2013). Genetic structure and association mapping of cold tolerance in improved japonica rice germplasm at the booting stage. *Euphytica* 193:369-382.

Davila Olivas NH, Kruijer W, Gort G, Wijnen CL, Loon JJA, Dicke M (2017). Genome-wide association analysis reveals distinct genetic architectures for single and combined stress responses in arabidopsis thaliana. New Phytologist 213:838-851.

Dong Z, Li D, Hu X, Liang L, Wu G, Zeng S, Liu E, Wu Y, Wang H, Bhanbhro L B, Dang X, Hong D (2018). Mining of favorable marker alleles for flag leaf inclination in some rice (Oryza Sativa, l.) accessions by association mapping. Euphytica 214:117.

Evanno G, Regnaut S, Goudet J (2005). Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. Molecular Ecology 14:2611-2620.

Excoffier L, Laval G, Schneider S (2005). Arlequin (version 3.0): an integrated software package for population genetics data analysis. Evolutionary Bioinformatics 1:47-50.

Fujino K, Matsuda Y, Ozawa K, Nishimura T, Koshiba T, Fraaije MW, Sekiguchi H (2008). NARROW LEAF 7 controls leaf shape mediated by auxin in rice. Molecular Genetics and Genomics 279:499-507.

Fujino K, Obara M, Shimizu T, Koyanagi K O, Ikegaya T (2015). Genome-wide association mapping focusing on a rice population derived from rice breeding programs in a region. Breeding Science 65:403-410.

Gladun IV, Karpov EA (1993). Distribution of assimilates from the flag leaf of rice during the reproductive period of development. Russian Journal of Plant Physiology 40:215-219.

Hardy OJ, Vekemans X (2002). SPAGeDi: a versatile computer program to analyse spatial genetic structure at the individual or population levels. Molecular Ecology Notes 2:618-620.

Huang X, Han B (2018). Genome-wide association mapping of complex traits in rice. In: Sasaki T., Ashikari M. (eds), Rice genomics, genetics and breeding. Springer, Singapore, pp. 497-510.

Jiang S, Zhang X, Wang J, Chen W, Xu Z (2010). Fine mapping of the quantitative trait locus qFLL9 controlling flag leaf length in rice. Euphytica 176:341-347.

Jin F, Hua S, Xu H, Yang L, Jiang Y, Xu Z, Shao X (2018). Comparisons of plant-type properties and grain quality in filial generations of Indica×Japonica hybridization grown in different rice-growing areas of China. International Journal of Agriculture and Biological Sciences 20:959-965.

Jin L, Lu Y, Xiao P, Sun M, Corke H, Bao J (2010). Genetic diversity and population structure of a diverse set of rice germplasm for association mapping. Theoretical and Applied Genetics 121:475-487.

Kanbe T, Sasaki H, Aoki N, Yamagishi T, Ebitani T, Yano M, Ohsugi R (2008). Identification of QTLs for improvement of plant type in rice (Oryza sativa L.) using Koshihikari/Kasalath chromosome segment substitution lines and backcross progeny F_2 population. Plant Production Science 11:447-456.

Khowaja FS, Price AH (2008). QTL mapping rolling, stomatal conductance and dimension traits of excised leaves in the Bala×Azucena recombinant inbred population of rice. Field Crops Research 106:248-257.

Kraakman ATW, Martínez F, Mussiraliev B, van Eeuwijk FA, Niks RE (2006). Linkage disequilibrium mapping of morphological, resistance, and other agronomically relevant traits in modern spring barley cultivars. Molecular Breeding 17:41-58.

Khush GS (1995). Breaking the yield frontier of rice. GeoJournal

35:329-332.
Laza MR, Kondo M, Ideta O, Barlaan E, Imbe T (2006). Identification of quantitative trait loci for $\delta^{13}C$ and productivity in irrigated lowland rice. Crop Science 46:763-773.
Liu CG, Zhou XQ, Chen DG, Li LJ, Li JC, Chen YD (2014). Natural variation of leaf thickness and its association to yield traits in indica rice. Journal of Integrative Agriculture 13:316-325.
Liu KJ, Muse SV (2005). PowerMarker: an integrated analysis environment for genetic marker analysis. Bioinformatics 21:2128-2129.
Liu C, Zhang G (2010). Genetic diversity revealed by SSR markers and temporal trends of major commercial inbred indica rice cultivars in South China in 1949-2005. Acta Agronomica Sinica 36:1843-1852.
Mackay I, Powell W (2007). Methods for linkage disequilibrium mapping in crops. Trends in Plant Science 12:57-63.
Matsushima S (1976). High-yielding rice cultivation. University of Tokyo Press http://agris.fao.org/agris-search/search.do?recordID=XF2015044376
Nakagawa S (2004). A farewell to Bonferroni: the problems of low statistical power and publication bias. Behavioral Ecology 15:1044-1045
Neumann K, Kobiljski B, Denčić S, Varshney RK, Börner A (2011). Genome-wide association mapping: a case study in bread wheat (*Triticum aestivum* L.). Molecular Breeding 27:37-58.
Pace J, Gardner C, Romay C, Ganapathysubramanian B, Lübberstedt T (2015). Genome-wide association analysis of seedling root development in maize (zea mays l.). BMC Genomics 16:47.
Panaud O, Chen X, McCouch SR (1996). Development of microsatellite markers and characterization of simple sequence length polymorphism (SSLP) in rice (*Oryza sativa* L). Molecular Genetics and Genomics 252:597-607.
Peng S (2000). Single-leaf and canopy photosynthesis of rice. In, Sheehy J E, Mitchell P L, Hardy B, eds. Redesigning Rice Photosynthesis to Increase Yield. IRRI, Los Bannos, Philipines pp. 213-228.
Pritchard JK, Stephens M, Donnelly P (2000). Inference of population structure using multilocus genotype data. Genetics 155:945-959
Qi J, Qian Q, Bu Q, Li S, Chen Q, Sun J, Liang W, Zhou Y, Chu C, Li X, Ren F, Palme K, Zhao B, Chen J, Chen M, Li C (2008). Mutation of the rice Narrow leaf1 gene, which encodes a novel protein, affects vein patterning and polar auxin transport. Plant Physiology 147:1947-1959.
Qu M, Zheng G, Hamdani S, Essemine J, Song Q, Wang H, Chu C, Sirault X, Zhua X (2017). Leaf photosynthetic parameters related to biomass accumulation in a global rice diversity survey. Plant Physiology 175:248-258.
Rode J, Ahlemeyer J, Friedt W, Ordon F (2012). Identification of marker-trait associations in the German winter barley breeding gene pool (*Hordeum vulgare* L.). Molecular Breeding 30:831-843.
Sabiel SAI, Huang S, Hu X, Ren X, Fu C, Peng J, Sun D (2017). SNP-based association analysis for seedling traits in durum wheat (triticum turgiduml.durum(desf.)). Breeding Science 67:83-94.
Sakamoto T, Morinaka Y, Ohnishi T, Sunohara H, Fujioka S, Ueguchi-Tanaka M, Mizutani M, Sakata K, Takatsuto S, Yoshida S, Tanaka H, Kitano H, Matsuoka M (2006). Erect leaves caused by brassinosteroid deficiency increase biomass production and grain yield in rice. Nature Biotechnology 24:105-109.
Shen B, Yu WD, Zhu YJ, Fan YY, Zhuang JY (2012). Fine mapping of a major quantitative trait locus, qFLL6.2, controlling flag leaf length and yield traits in rice (Oryza sativa L.). Euphytica 184:57-64.
Shinada H, Yamamoto T, Yamamoto E, Hori K, Yonemaru J, Matsuba S, Fujino K (2014). Historical changes in population structure during rice breeding programs in the northern limits of rice cultivation. Theoretical and Applied Genetics 127:995-1004.
Takai T, Kondo M, Yano M, Yamamoto T (2010). A Quantitative Trait Locus for Chlorophyll Content and its Association with Leaf Photosynthesis in Rice. Rice 3:172-180.
Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S (2011). MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Molecular Biology and Evolution 28:2731-2739.
Wang P, Zhou G, Yu H, Yu S (2011). Fine mapping a major QTL for flag leaf size and yield-related traits in rice. Theoretical and Applied Genetics 123:1319-1330.
Wang R, Leng Y, Ali S, Wang M, Zhong S (2017). Genome-wide association mapping of spot blotch resistance to three different pathotypes of Cochliobolus sativus, in the USDA barley core collection. Molecular Breeding 37:44.
Wang S, Basten CJ, Zeng ZB (2007). Windows QTL cartographer 2.5. Department of Statistics, North Carolina State University, Raleigh, NC. http://statgen.ncsu.edu/qtlcart/WQTLCart.htm
Xu Q, Yuan X, Wang S, Feng Y, Yu H, Wang Y, Yang Y, Wei X, Li X (2016). The genetic diversity and structure of indica rice in China as detected by single nucleotide polymorphism analysis. BMC Genetics 17:53.
Yang SR, Zhang LB, Wang JM (1984). The theory and method of ideal plant morphology in rice breeding. Scientia Agricultura Sinica 17:6-13.
Yang X, Yan J, Shah T, Warburton M, Li Q, Li L, GaoY, Chai Y, Fu Z, Zhou Y, Xu S, Bai G, Meng Y, Zheng Y, Li J (2010). Genetic analysis and characterization of new maize association mapping panel for quantitative trait loci dissection. Current Science 121:417-431.
Yu J, Buckler ES (2006). Genetic association mapping and genome organization of maize. Current Opinion in Biotechnology 17:155-160.
Yu JM, Pressoir G, Briggs WH, Bi IV, Yamasaki M, Doebley JF, McMullen M, Gaut BS, Nielsen DM, Holland JB, Kresovich S, Buckler ES (2006). A unified mixed-model method for association mapping that accounts for multiple levels of relatedness. Nature Genetics 38:203-208.
Yu P, Yuan X, Xu Q, Wang C, Yu H, Wang Y, Tang S, Wei X (2013). Genetic Structure and Indica/Japonica Component Changes in Major Inbred Rice Varieties in China. Rice Science 20:39-44.
Yuan LP (1997). Hybrid rice breeding for super high yield. Hybrid Rice 12(6):1-6.
Zhai L, Zheng T, Wang X, Wang Y, Chen K, Wang S, Wang Y, Xu J, Li Z (2018). QTL mapping and candidate gene analysis of peduncle vascular bundle related traits in rice by genome-wide association study. Rice 11:13.
Zhang P, Li J, Li X, Liu X, Zhao X, Lu Y (2011). Population structure and genetic diversity in a rice core collection (Oryza sativa L.) investigated with SSR markers. PLoS One 6:e27565.
Zhang P, Liu X, Tong H, Lu Y, Li, J (2014). Association mapping for important agronomic traits in core collection of rice (oryza sativa l.) with ssr markers. PLoS One 9:e111508.
Zhao WG, Chung JW, Kwon SW, Lee JH, Ma KH, Park YJ (2013). Association analysis of physicochemical traits on eating quality in rice (*Oryza sativa* L.). Euphytica 191:9-21.
Zhao X, Xu J, Zhao M, Lafitte R, Zhu L, Fu B, Gao Y, Li Z (2008). QTLs affecting morph-physiological traits related to drought tolerance detected in overlapping introgression lines of rice (Oryza sativa L.). Plant Science 174:618-625.
Zheng K, Huang N, Bennett J, Khush GS (1995). PCR-based marker-assisted selection in rice breeding. IRRI discussion paper series No.12. Manila: IRRI
Zhou Y, Fang YX, Zhu JY, Li SQ, Gu F, Gu MH, Liang GH (2010). Genetic analysis and gene fine mapping of a rolling leaf mutant (rl11(t)) in rice (Oryza sativa L.). Chinese Science Bulletin 55:1763-1769.
Zhu C, Gore M, Buckler ES, Yu J (2008). Status and prospects of association mapping in plants. Plant Genome 1:5-20.
Zhu G, Peng S, Huang J, Cui K, Nie L, Wang F (2016). Genetic Improvements in Rice Yield and Concomitant Increases in Radiation- and Nitrogen-Use Efficiency in Middle Reaches of Yangtze River. Scientific Reports 6:21049.

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Genotype and environment interaction effect on yield and yield components in Desi-type Chickpea [*Cicer arietinum* (L.)]

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Chickpea is the major pulse crop cultivated in Ethiopia. However, its production is constrained due to genotype instability and environmental variability. This research was carried out to examine the magnitude of environmental effect on yield of chickpea genotypes and to investigate the stability and adaptability of genotypes under different agro-ecologies. Twelve genotypes evaluated in randomized complete block design with three replications in three locations for two continuative years. Various stability indices used to assess stability and genotype by environment performances. Combined analysis of variance for yield and yield components revealed highly significant (P≤0.01) differences for genotypes, environments and their interaction. Growing years do not show difference. The significant interaction showed genotypes respond differently across environments. At Guduru, Hareto and Gitilo, top performing genotype in grain yield were genotype 229961 (2.33ton/ha), genotype 225887 (3.6ton/ha), and genotype 225887 (2.23/ha), respectively. The first two principal components (PC1 and PC2), which were used to create a two-dimensional bi-plot, explained 7.5 and 3.75% of AMMI sum of squares, respectively. Hareto and Guduru are the most differentiating environments, while Gitilo is more responsive environment than the other environments since it is far away from the other in altitude. Genotype 212476 and 212976 were the most stable as well as productive at Hreto environment, genotype 229961 and 225887 were the most stable as well as productive at Guduru environment. Genotype 229959 and 215189 were stable with intermediate productive in both years and at three of the growing locations. Genotype 219804 and 225889 are less responsive. The best genotypes with respect to Guduru site are 229959 and 215189: the best genotype for environments with respect to Hareto was 212476 and 212976, and to Gitilo environment 229961 and 225887 genotypes.

Key words: Additive main effects and multiplicative interaction (AMMI), Desi-type, interaction, AMMI stability value (ASV).

INTRODUCTION

A diploid (2n=16 chromosomes) and self-pollinated plant, chickpea is a temperate pulse crop, probably originated in Southeastern Turkey and spread to other parts of the world. Crop improvement efforts have improved adaptation

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of chickpea to warmer conditions in the subtropics. Chickpea is frequently divided for breeding purpose into two main types' desi and Kabuli. This distinction is mainly made on the basis of seed size and color. Desi showing small (1500 seeds/lb) and colored seeds while the Kabuli types with large (800 seeds/lb) and white seeds (Singh, 1987; Moussa et al., 2000). It is believed that the Desi type originates first and the Kabuli type originated later through natural mutation. Although both types differ in many traits, they cross easily with each other and the transfer of genes from one type to another is easy (Singh and Saxena, 1999). Chickpea is an important source of protein in the diets of the poor in the SAT and WANA regions, and is particularly important in vegetarian diets. In addition, it is being used increasingly as a substitute for animal protein.

Environmental factors such as soil moisture, sowing time, fertility and temperature and day length have strong influence during various stages of plant growth (Bull et al., 1992). The environment is changing day-by-day and this implies that it is necessary to evaluate crop genotypes at different locations to assess their performances. One approach to improve the chickpea yield is to identify stable genotypes that perform consistently better under diverse environments (Ghulam et al., 2012). The performance of a genotype is not always the same in different locations as it influenced by environmental factors. To assess yield stability among varieties, multi-location trials with appropriate stability analysis method is required. Differences in genotype stability and adaptability to environment can be qualitatively assessed using the bi-plot graphical representation that scatters the genotypes according to their principal component values (Vita et al., 2010).

In Ethiopia, especially in Horo Guduru Wollega area, there is no sufficient information on the genotype by environment interaction effects on yield and yield related traits of chickpea. Therefore, the current research was undertaken to examine the magnitude of environmental effect on yield and yield related traits of Desi-type chickpea genotypes, to study the nature and extent of genotype by environment interaction on seed yield of Desi-type -chickpea genotypes.

MATERIALS AND METHODS

The experiment was conducted during the 2015 and 2016 main cropping season at three locations representing various chickpea growing agro-ecologies of Horo Guduru Wollega Zone, Western Ethiopia. The environments were Guduru, Gitilo and Hreto. Twelve (12) Desi-type chickpea genotypes were included in the study (Table 1). The plant materials were obtained from Ethiopian Biodiversity Institute. Planting of the genotypes was done in early and mid-August up to first week of September depending on moisture duration of each environment using randomized complete block design with three replications at each site under rain fed conditions (Table 2). Each genotype was planted in four rows of 2 m length and at 1.2 m width. A spacing of 30 cm row to row distance and 10 cm plant to plants were used on a plot size of 2.4 m^2. Recommended fertilizer type and rate was applied. Weeding and other management practices were done as required for each site. Data were recorded on days to 50% flowering, 90% physiological maturity, plant height, the number of pods per plant, the number of seeds per plant, number of primary branches per plant, and grain yield in kg per plot and then converted to ton per hectare. The collected data was analysed using SAS V.9.2 for Combined analysis, Genst.13th edition (SP2) for additive main effects and multiplicative interaction (MMI) and AMMI stability value (ASV).

Analysis of variance

From the combined analysis of variance, the mean squares due to genotypes, environments, year, genotype by environment interaction, genotype by year interaction, environment by year interaction and genotype by environment and by year interaction were highly significant for the traits, days to flowering, days to maturity, plant height, number of pods per plant and number of pod bearing branches per plant. However, there were no-significant effects of all these three sources of variation on the number of seeds per pod (Table 3). The separate analysis of variance for all yield related traits, except for number of seeds per pod at each location exhibited highly significant ($P \leq 0.01$) differences among Desi-type chickpea genotypes for the days to flowering, days to maturity, number of pods per plant, plant height, and number of pods bearing branches per plant at all locations. Similar results were reported by different researchers who worked on chickpea (Singh et al., 1990; Bozoglu and Gulumser, 2000; Vargas et al., 2007). The responses of genotypes in terms of all yield related traits were different both within and across locations. This indicated that the efficiency of a breeding program aimed at yield improvement is impaired due to genotype by environment interaction, which complicates the process of crop variety development especially when varieties are selected in one environment and used in others (Ahmad et al., 2011).

Significant effects observed for plant height, number of pods per plant not only for genotypes but also for locations, year, and genotype by environment interaction, genotype by year interaction, environment by year interaction and genotype by environment and by year interaction, reflecting genetic variability in experimental material as well as difference in the environmental conditions even through the two continuative growing years (Table 3). Averaged over all genotypes the highest plant height was recorded at Hareto (49.7 cm) and the shortest was at Gitilo (33.6 cm) (Table 6). Number of pods per plant is an important selection criterion for the development of high yielding genotypes and strongly influenced by environment in chickpea (Malik et al., 1988). Marked variation was observed in the performance of genotypes over the three locations (Table 3). Number of pods per branch was highest at Hareto (10.5) and least at Guduru (2.5). The genotypes mean values for number of pods per branch varied from 4.52 for genotype 215189 to 7.2 for genotype 225887. The highest mean number of pods per branch was recorded for genotypes 225887 (11) followed by 229961 (9.5) and 212916 (9) in Table 2. These results are consistent with the findings of Singh and Bains (1984) and Malik et al. (1988). These results indicate variability for number of pods per branch and its sensitiveness to environmental fluctuations.

RESULTS AND DISCUSSION

Performance of genotypes on grain yield

The combined analysis of variance (Table 3) for grain yield exhibited significant ($P \leq 0.01$) effects of locations,

Table 1. Location and descriptions of weather conditions for the three tested locations.

Location	Annual temperature (°C)	Annual rainfall (mm)	Locatation	
			Altitude (m)	Latitude
Gitilo	Min 9.24 - max 23.28	1844.19	2854	09° 12'N/37° 0'E 28.7'
Guduru	Min 16.60 - max 26.19	1816.86	2265	09° 04' N/37° 0'E 176'
Hareto	Min 14.60 - max 23.19	1832.06	2485	09° 04' N/37° 0'E 19.64'

Table 2. Experimental material (Desi-type chick-pea genotypes) used in the experiment.

Entry No.	Genotype code	Source	Year
1	208900	IEBC	2015
2	215188	IEBC	2015
3	212476	IEBC	2015
4	212916	IEBC	2015
5	229959	IEBC	2015
6	219804	IEBC	2015
7	230795	IEBC	2015
8	229961	IEBC	2015
9	215189	IEBC	2015
10	208977	IEBC	2015
11	225887	IEBC	2015
12	225889	IEBC	2015

genotypes and genotype by environment interaction, indicating differences in environments, the presence of genetic variability among genotypes and year. Various authors (Singh et al., 1990; Bozoglu and Gulumser, 2000) reported the presence of significant genotype by environment interaction in chickpea. The overall mean yield of the location varied from 0.78 to 2.66 ton per hectare (Table 4) and thus, the three environments showed wide variation in yield potential. The highest mean grain yield was obtained at Hareto (3.13 ton/ha) and the lowest was from Gitilo (0.75 ton/ha). The possible reason was that late planting was done at Gitilo and due to this moisture; stress occurred at vegetative and pod setting stage while relatively sufficient moisture was available at Hareto. Genotypic means across the locations (mean environmental index) indicated that maximum mean grain yield across all the three locations in two year were obtained from 225887 genotype (2.68 ton/ha) and the minimum was from genotype 219804 (0.78 ton/ha). Genotype by environment interaction causes differences in yield rank of genotypes in different locations; thus, it becomes important for the chickpea breeders in terms of selection efficiency and genotype suggestions for different locations.

Genotypes showed inconsistent yield performances across all environments. Genotypes expressed their genetic potential differently in different environments (Table 4). At Guduru, Hareto and Gitilo the top performing genotypes were 225887 (2.36 ton/ha), 229961 (3.4 ton/ha) and 225887 (2.33 ton/ha) at the first growing year (2015) and Genotype 215189 (2.7 ton/ha) genotype 225887 (3.43 ton/ha) and genotype 212916 (2.41 ton/ha) in the second year (2016), respectively. Genotype 225887 tops performing at Hareto and Guduru in both years in average with the average mean yield of 3.12 and 2.7 ton/ha, respectively and it is a rich (potential environment) genotype whereas genotype 219804 performs poorly to all location through both growing seasons, therefore called a genotype with poor environment. The mean grain yield averaged over environments, year and genotypes were 1.78 ton/ha (Tables 3 and 4). In summary, the relative ranking of genotypes at all the three environments were different and CV values of genotype ranged from 2.1 to 18.1% (Table 6).

The AMMI analysis of variance of grain yield of 12 Desi-type chickpea genotypes tested in three environments isshown in Table 5. The analysis revealed that Desi-type chickpea genotypes were significantly ($P \leq 0.01$) affected by environments (E), genotypes (G), Year(Y), genotype by environment interaction, Year by Genotype and Year by Environment by Genotype interaction. The main effects of environment and genotype accounted for 32.8 and 24.5%, respectively,

Table 3. Mean square Analysis of variance for the six yield related characters for Desi type chickpea genotypes.

Var	Gen	Year	Env	G×E	G×Y	Y×E	G×Y×E	Err	M	CV%
Df	11	1	2	222	11	2	22	142	-	-
DF	99*	661*	300*	21.6*	23*	45*	41*	6.8	59	5
DM	522*	1345*	130*	27.8*	27.3*	1343*	28.5*	9.3	101	2.1
PH	225*	340*	590*	21.2*	27*	5817*	111*	12.3	39	9
BpP	13.9*	0.5	3*	0.4	0.6	24.6*	5	0.5	3.6	18.1
PpB	19*	184*	1.5	10.6*	1.7	582*	9.2*	2	6	14
SpP	0.99	0.077	0.12	0.6	0.12	0.06	0.2	1.47	1.7	12
GY	3.3*	0.08*	11*	3.6*	0.3	10.12*	7.6*	0.3	1.75	11.7

GY=Grain yield, DF=days to 75% flowering, DM=days to 75% maturity, PH=plant height, BpP=number of pod bearing branches per plant, PpB=number of pod per branches and SpP= number of seed per pod. Gen= genotypes, Y= year, Env=environment, G×E=genotype by environmental interaction, Y×E=year and environment interaction, Y×G= year and genotype interaction, Y×E×G=interaction of year, environment & genotype, CV=coefficient of variation.

Table 4. Mean performance of the genotypes in grain yields in ton/hactar at three of the locations.

Genotype	Guduru	Hareto	Gitilo	Mean
208900	1.58	2.6	1.1	1.5
215188	1.35	2.75	1.28	1.6
212476	1.7	2.7	1.16	1.67
212916	1.9	2.76	1.41	1.8
229959	2.06	2.75	1.12	1.89
219804	0.93	0.9	0.79	0.87
230795	1.4	2	1.3	1.2
229961	2.33*	3.03*	1.46	2.28
215189	2.2	2.87	1.43	2
208977	1.2	2.4	0.92	1.47
225887	2.25	3.13*	2.3*	2.6*
225889	1.06	2.3	1.16	1.48
Mean	1.67	2.58	1.3	1.7
CV%	9.1	7.6	8.5	12
LSD5%	0.83	0.75	0.45	0.35

*Guduru, Hareto & Gitilo=are environments, LSD=least significant difference, CV=coefficients of variation.

and G × E interaction accounted for 19.2% of the total variation of genotype by environment on data for grain yield indicating environment had larger effect for its variability. G×E×Y also accounts for 19.7% of the total variation for Desi-type chickpea genotypes. However, this variation is not due to year but due to the contribution effect of genotype and environment. The first two principal components (PC1 and PC2), which were used to create a two-dimensional bi-plot, explained 89.4 and 6.5% of AMMI sum of squares, respectively. According to the AMMI model, the genotypes, which characterized by means greater than grand mean and the IPCA, score nearly zero are considered as generally adaptable to all environment (Ezatollah et al., 2013). However, the genotype with high mean performance and with large value of IPCA score is considered as having specific adaptability to the environments. The large sum of squares for environments showed that the environments were diverse, with large differences among environmental means causing most of the variation in grain yield. This is in synchronization with the findings of Singh et al. (1990), Yan (2002) and Yan and Tinker (2006) in chickpea production. This result also indicates the considerable influence of environments on the yield performance of Desi-type chickpea genotypes in Horo Guduru Wollega Zone. The magnitude of the genotype by environment sum of squares was more than two times that for genotypes and year, indicating that there were considerable differential genotype responses across environments rather than year.

Table 5. AMMI analysis of variance for branch per plant, pod per branch and grain yield in ton/hectare.

Var	Df	B/Plant			Pod/branch			Grain yield(ton/hac)		
		SS	MS	SS%	SS	MS	SS%	SS	MS	SS%
Tot		400.8	-	-	2059	-	-	112	-	-
Gen	11	60.3	13.9**	15	212.8	19.3*	10.3	27.3	2.4*	24.5
Year	1	0.6	0.5	0.2	184	184.6**	8.9	3.7	3.6*	3.3
Env	2	49	24.9*	24.4	22.9	12.4*	56.8	22.2	11**	32.8
G×E	22	99.7	4.5*	22.9	1165	52.9**	6.6	36	21.6**	19.2
G×Y	11	6.2	0.6	1.6	18.2	1.7*	1.2	1.4	0.3	1.2
Y×E	2	8.9	4.6*	2.2	9.4	4.7*	0.45	2.4	1.2*	13.4
G×Y×E	22	101	5*	25.5	202	9.2**	9.8	22.7	1.55*	19.7
PCI1	12	19.76	1.8*	4.9	7.8	0.62	0.37	8.3	0.7*	7.4
PCI2	10	0.89	0.02	0.09	1.6	0.26	0.13	4.2	0.42*	3.75
Err	142	7.4	0.5	1.12	19.8	2	7	10.3	0.23	1.6

GY=Grain yield, DH=days to 75% flowering, DM=days to 75% maturity, PH=plant height, BpP=number of pod bearing branches per plant, PpB= number of pod per branches and SpP=number of seed per pod. Gen=genotypes, Y=year, Env=environment, G×E=genotype by environmental interaction, Y×E=year and environment interaction, Y×G=year and genotype interaction, Y×E×G= interaction of year, environment & IPC1=the first principal component and IPC2= the second principal component.

Table 6. Genotype, environment and year G/Y means and scores ASV.

Genotype	Genotype mean	IPCAg[1]	IPCAg[2]	ASV
208900	1.515	0.10963	0.09800	0.33
215188	1.607	0.02568	0.14697	0.15
212476	1.664	0.07454	0.18151	0.124
212916	1.803	-0.01127	0.22023	0.12
229959	1.899	0.28170	-0.51571	0.5
219804	1.050	-0.57628	-0.16883	0.59
230795	1.297	-0.37405	0.17166	0.4
229961	2.289	0.49426	0.10729	0.09
215189	2.051	-0.02130	-0.42623	0.4
208977	1.474	0.02164	0.11215	0.1
225887	2.679	0.12476	0.12034	0.07
225889	1.481	-0.14932	-0.04740	0.13
Environment	**Env. mean**	**IPCAe[1]**	**IPCAe[2]**	
Guduru	1.3	0.057	-0.66	
Hareto	2.064	0.62	0.37	
Gitilo	1.7	-0.68	0.29	
Year	**Year mean**	**IPCAy(1)**	**IPCAy(2)**	
2015	1.84	-0.43	0.002	
2016	1.59	0.438	0.000	

The AMMI I, bi-plot for grain yield of the 12 Desi-type chickpea genotypes at three environmental conditions for two consecutive years is as shown in Figure 1. The main effects (genotypes, environments and year) accounted for 95.9% of the total variation and IPCA 1 accounted for 89.4% of the total variation due to genotype by environment interaction alone. Environments showed high variation in both main effects and interactions (IPCA1) (Figure 1). Hareto is the most favorable environments; Gitilo is the least favorable environments, while Guduru is the averaged environment.

All environments are almost the same based on their

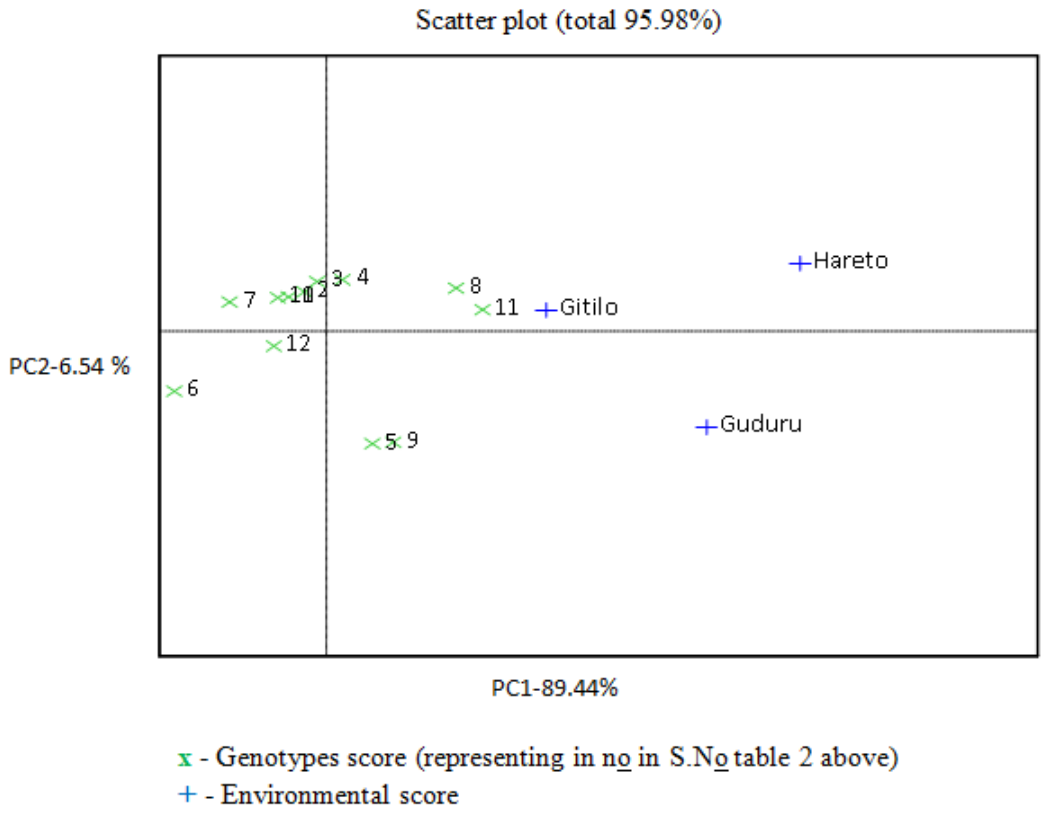

Figure 1. AMMI bi-plot analysis of IPCA scores genotype and environment means for Desi-type genotypes.

IPCA 1 scores. Hareto and Gitilo are in quadrant I and have got large positive IPCA1 scores, which interact positively with genotypes that have positive IPCA1 scores and negatively with those genotypes having negative IPCA1 scores. Guduru in quadrants IV and have got small positive IPCA1 scores, which interact positively with genotypes that have positive IPCA1 scores and negatively with those genotypes having negative IPCA1 scores (Figure 1). The environments can be sub-grouped according to their average yield over the genotypes. According to environmental IPCA1 scores, Hareto and Gitilo were more stable and had lower genotype by environment interaction, and had high yield performance. On the other hand, the highest IPCA1 scores belonged to Hareto and Guduru, and they had high yield performance. According to IPCA1, environment Hareto was an ideal environment for selecting genotypes with specific adaptation to high input conditions.

Genotypes that fall near the origin are relatively wider adapted while genotypes that fall far from the origin are most probably specific adaptors. In Figure 1, the genotypes and locations that are located far away from the origin are more responsive. Hareto and Guduru are the most differentiating environments, while Gitilo is more responsive environment than the other environments since it is near to the origin. Genotypes 212476, 212976, 229961 and 225887 were the most stable as well as productive. Genotypes 229959 and 215189 were stable with intermediate productivity. 219804 and 225889 are less responsive. Genotypes and environments that fall into the same sector interact positively; negatively if they fall into opposite sectors (Osiru et al., 2009). A genotype showing high positive interaction in an environment obviously has the ability to exploit the agro-ecological or agro-management conditions of the specific environment. If they fall into adjacent sectors, interaction is somewhat more complex. In this case, the best genotypes with respect to Guduru site are 229959 and 215189; the best genotype for environments with respect to Hareto and Gitilo is 212476, 212976, 229961 and 225887. Genotypes 219804 and 225889 respond negatively to all Hareto, Guduru and Gitilo environments.

The AMMI stability (ASV) value is the distance from zero in a bi-plot between the IPCA 1 scores and the IPCA 2 scores (Getachew et al., 2015). In the AMMI analysis, the IPCA 1 score contributes more to the genotype-environment (GE) interaction sum of squares. Thus, the relative contribution of IPCA 1 and IPCA 2 to the total G × E interaction sum of squares has to be weighted by the proportional difference between IPCA 1 and IPCA 2

scores.

Genotypes 208977, 225889, 225887, 215188 and 212476 had little interaction because of their weakest reaction to IPCA 2. Similarly, their little interaction was confirmed by their least AMMI stability value and thus, better stability in yield across environments. From thus genotypes some were the highest yielder of all. In addition to their greater interaction (strong reaction to IPCA 2), genotypes 229959, 219804 and 229961; however, showed high AMMI stability values, reflecting unstable in yield performance across environments (Table 5). Although no genotype was superior in all the test environments, the genotypes 208900, 212476 and 230795 were stable across environments both in their mean performance and ASV.

Conclusion

Genotype by environment interaction causes differences in yield rank of genotypes in different locations; thus, it becomes important for the chickpea breeders in terms of selection efficiency and genotype suggestions for different locations. Important approach to improve the chickpea yield is to identify stable genotypes that perform consistently better under diverse environments (Ghulam et al., 2012). The performance of a genotype is not always the same in different locations as it influenced by environmental factors. To assess yield stability among varieties, multi-location trials with appropriate stability analysis method is required. Differences in genotype stability and adaptability to environment can qualitatively assessed using the bi-plot graphical representation that scatters the genotypes according to their principal component values.

From the combined analysis of variance, the mean squares due to genotypes, environments, year, and their interactions were highly significant for all the traits studied. However, there were no-significant effects of all these three source of variation on the number of seeds per pod. The separate analysis of variance for all yield related traits, except for number of seed per pod at each location exhibited highly significant (P≤0.01) differences among Desi-type chickpea genotypes for the days to flowering, days to maturity, number of pods per plant, plant height, and number of pod bearing branches per plant at all locations. Significant difference due to genotypes showed inconsistent performances across all environments and expressed their genetic potential differently in different environments. The significant effect shown in G×E indicated genotypes need separate evaluation for each location. In the other way, the non-significant effect observed for the two growing season depicts there were no more micro-environmental variation at both season at the locations. But, the results of some genotypes showing little variant in yield result through growing season tell as there may be some preference of these genotypes to even little micro-climate. The mean grain yield averaged over environments, year and genotypes were 1.78 ton/ha (Tables 3 and 4). In summary, the relative ranking of genotypes at all the three environments were different and CV values of genotype ranged from 2.1 to 18.1%. The AMMI analysis of variance for grain yield of 12 Desi-type chickpea genotypes tested in three environments were significantly (P≤0.01) affected by Environments (E), Genotypes (G), Year, Genotype by Environment interaction, Year by Genotype and Year by Environment by Genotype. The main effects of E and G accounted for 19.8 and 24.5%, respectively, and G × E interaction accounted for 32.2% of the total variation of genotype by environment data for grain yield. G×E×Y also accounts 19.7% of the total variation for Desi-type chickpea genotypes. However, this variation is not due to year but due to the contribution effect of G and E. The first two principal components (PC1 and PC2), which were used to create a two-dimensional bi-plot, explained 7.5 and 3.75% of AMMI sum of squares, respectively. According to the AMMI model, the genotypes, which is characterized by means greater than grand mean and the IPCA, score nearly zero are considered as generally adaptable to all environment (Ezatollah et al., 2013). However, the genotype with high mean performance and with large value of IPCA score are consider as having specific adaptability to the environments.

In AMMI stability (ASV) value, some genotypes had little interaction because of their weakest reaction to IPCA 2. Similarly, their little interaction was confirmed by their least AMMI stability value and thus, better stability in yield across environments. Thus, some genotypes were the highest yielder of all.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

REFERENCES

Ahmad B, Ial ha, Shahid RM, Asif M, Muhammad I, Ramatullah Q (2011). Grain yield stability in chickpea (cicer arietinum l.) Across environments. Pakstan Journal of Botany 43(5):2947-2951.

Bozoglu H, Gulumser A (2000). Determination of genotype by environment interaction of some agronomic characteristics in dry bean (Phaseolus vulgaris L). Turky Journal of Agriculture 24:211-222

Bull JK, Cooper M, Delacy IH, Basford KE, Woodruff DR (1992). Utility of repeated checks for hierarchical classification of data from plant breeding trials. Field Crop Research 30:79-95.

Ezatollah F, Mahnaz R, Mohammad MJ (2013). AMMI analysis of phenotypic stability in chickpea genotypes over stress and non-stress environments. International Journal of Agriculture and Crop Science 5(3):253-260.

Getachew T, Firew M, Asnake F, Million E (2015). Genotype x environment interaction and stability analysis for yield and yield related traits of Kabuli-type Chickpea (*Cicer arietinum* L.) in Ethiopia. African Journal of Biotechnology 14(18):1554-1575.

Ghulam S, Adeel S, Zahid A, Talat M, Ahmad B, Ijaz RN (2012). Stability analysis of yield and yield components in chickpea (*cicer*

arietinum l.) Genotypes across three rain fed locations of Pakistan. Pakistan. Journal of Botany 44(5):1705-1709.
Malik BA, Hussain SA, Zahid MA (1988). Grain legume status in agriculture. Progress in Farming 3:23-26.
Moussa EH, Millan T, Moreno MT, Gil J (2000). Genetic analysis of seed size, plant height, day to flower and seed/plant by using both morphological and molecular markers in chickpea. Journal of Genetics and Breeding 54:101-107.
Osiru MO, Olanya OM, Adipala E, Kapinga R, Lemaga B (2009). Yield stability analysis of Ipomoea batatus L. cultivars in diverse environments. Australian Journal of Crop Science 3(4):213-220.
Singh H, Bains KS (1984). Stability analysis of varietal mixtures in chickpea (*Cicer arietinum* L.). Z. Pflanzenzuecht 92:159-167.
Singh KB, Bejiga G, Malhotra RS (1990). Associations of some characters with seed yield in chickpea collections. Euphytica 49:83-88.
Singh KB, Saxena MC (1999). Chickpeas. MaCmillan Education Ltd, London. 134p.
Singh KB (1987). Chickpea breeding. pp 127- 162. In: M.C. Saxena and K.B, Singh (eds). The chickpea. C.A.B. International, Wallingford, UK.
Vita PD, Mastrangeloa AM, Matteua L, Mazzucotellib E, Virzi N, Palumboc M, Stortod ML, Rizzab F, Cattivelli L (2010). Genetic improvement effects on yield stability in durum wheat genotypes grown in Italy. Field Crop Research 119:68-77.
Vargas M, Crossa J, Eeuwijk FA van, Ramirez ME, Sayre KD (2007). Using partial least square regression, factorial regression and AMMI models for interpreting Genotype x Environment interaction. Crop Science 39:955-967.
Yan W (2002). Singular value partitioning in biplot analysis of multienvironment trial data. Agronomy Journal 94:990-996.
Yan W, Tinker NA (2006). Biplot analysis of multi-environment trial data: Principles and applications. Canada Journal of Plant Science 86:623-645.

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