

## GROWTH AND P UPTAKE OF MAIZE AT VARIOUS P LEVELS UNDER AMF INOCULATION AND DIFFERENT PLANTING METHODS

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### **Abstract**

Immobility of phosphorus (P) in soil and its poor availability to crops is a matter of serious concern as it severely affects crop growth, yield and net income of farmers. Application of arbuscular mycorrhizal fungi (AMF) and suitable planting method (PM) may enhance P availability to crops. Experiments were conducted to investigate the growth and P uptake of maize in the presence of AMF and different PM during two consecutive years i.e. 2016 and 2017 at the University of Agriculture Research Farm Peshawar-Pakistan. Three different PM, viz. flatbed, raisedbed and ridges sowing, five levels of P (as  $P_2O_5$ ) (0, 30, 60, 90 and 120 kg/ha), with or without AMF inoculation were used. Higher plant stature (PS), leaf number per plant (L/P), leaf area index (LAI), SPAD value, crop growth rate (CGR) and absolute growth rate (AGR) of maize were achieved with the application of P @ 60 kg/ha. However higher specific leaf area (SLA) was achieved with P @ 90 kg/ha and total P uptake (TPU) with P @ 120kg/ha. PS, L/P, LAI, SPAD value, SLA, CGR, AGR and TPU were significantly higher with the application of AMF to the field. Higher PS, L/P, SPAD value, CGR and ABR were recorded with both raisedbed and ridge PM. Higher SLA and TPU were recorded with raisedbed PM while higher LAI was recorded with ridge PM. It was concluded that AMF application and P @ 60 kg/ha improved growth of maize. TPU is improved with AMF application and P application @ 120 kg/ha. Among the planting methods, both raisedbed and ridge planting methods improved growth of maize.

**Key words:** Maize (*Zea mays* L.), AMF inoculation, Planting methods, Phosphorus levels and Uptake, Growth.

### **Introduction**

Maize (*Zea mays* L.) is an important cereal crop of the world, ranks third following rice (*Oryza sativa*) and wheat (*Triticum aestivum*), and is cultivated in irrigated and high rainfall areas. It is an annual short day & C4 plant (Ali *et al.*, 2018). It is grown for food, as fodder in summer and autumn, and as an industrial crop (Sharif *et al.*, 2012). Maize is ranked as an important staple cereal crop in the developing countries including Pakistan, where over growing populations is facing shortage of food supplies.

Despite the fact that Pakistan has favourable climatic conditions for maize production, its yield is so far very low in Pakistan especially in its north western province of Khyber Pakhtunkhwa (KP). Average yield of maize in the USA was 9970 and in Canada it was 9588 kg/ha during 2014. In Pakistani Punjab, average yield of maize was 6132 kg/ha while in KP it was only 1927 kg/ha during 2016 (MNFSC, 2017). There are several reasons of low grain yield of maize in KP; the most important among which include low soil fertility, inadequate dose of phosphorus application, and inappropriate planting method.

Among the agro-management practices, suitable planting method is very important for improving crop productivity. Suitable planting method ensures better water and nutrient supply through improved root development resulting in better crop growth and productivity (Singh, 2011). Besides, suitable planting method maintain better plant population by ensuring maximum emergence and enabling the young plant to utilize the available resources more effectively, which further emphasis on the choice of improved method for planting (Quanqi *et al.*, 2008), or increasing productivity (Amin *et al.*, 2006). Hard soil surfaces as in flat bed PM, limit root growth, resulting in shorter root development which tend to concentrate near soil surface; hence plants

have excess to limited volume of soil for water and nutrients absorption (Chassot & Richner, 2002) resulting in reduced leaf expansion (Young *et al.*, 1997) and ultimately poor crop productivity. On the other hand porous and weed free soil, better aeration and light penetration, water movement and well-developed root system in ridge planting and raisedbed sowing enhance crop productivity (Khan *et al.*, 2012).

Phosphorus (P) being macronutrient, plays a very important role in numerous biochemical and physiological processes in plants. P produces strong cereal straw enhances plant roots and flower development, seed formation and crop maturity (Ibrahim & Kandil, 2007). In majority of cropping system, availability of P to plants is very low (Shenoy & Kalagudi, 2005). The availability of P is one of the most significant determinants in plant growth (Razaq *et al.*, 2017). Its deficiency can adversely affect the crop growth and productivity which may result yield losses (Raghthama & Karthikeyan, 2005). Most of soils in Pakistan are Phosphorus deficient (Wahid *et al.*, 2016). Therefore, Phosphorus fertilizers must be applied in sufficient amount for enhancing maize production (Rashid & Memon, 2001). Phosphate fertilizer is also one of the most expensive inputs in agriculture (Chaudhary, 2013); and need to be efficiently utilized particularly when resource poor farmers are unable to afford these expensive fertilizers. High purchasing cost of phosphate fertilizers in the country urges to find some alternate and cheap sources and methodologies to reduce the cost of production and improve the efficiency of the applied fertilizers.

Soil microbial community (including bacteria, fungi, microfauna and mesofauna) has an important role in production system (Sylvia *et al.*, 2005) by improving soil fertility and consequently the productivity. Fungi make mycorrhizal association with roots of higher plants, which functions as a bridge for energy flow and matter between

plants and soil (Siddiqui & Pichtel, 2008). Arbuscular Mocorrhizal Fungi (AMF) increase the effectiveness of absorbing capability of host roots as much as ten times. P being an immobile nutrient in soil, cannot diffuse easily, hence roots are deprived of this important element in their rhizosphere. Plants and AMF symbiosis results in an increase in plant growth mainly due to efficient uptake of P mineral by AMF hyphae (Harley & Smith, 1983). AMF hyphae extend into the soil, penetrate into deeper nutrient zone and increase the uptake efficiency of host plants, roots for immobile nutrients elements like P, Zn, Cu, S, Fe, Ca, Mg and Mn (Abdul-Malik, 2000). AMF inoculated plants have been proved to be more resistance to abiotic and biotic stresses, thus giving AMF an eco-friendly and a bio-fertilizing status. AMF make important natural component of the soil system (Kowalska *et al.*, 2015). The use of synthetic fertilizer can be considerably decreased by inoculating plants, thus ensuring optimum uptake of essential minerals by plant roots from the soil, resulting in even higher plant yield (Abbot and Robson, 1991). In olericulture and floriculture, the use of AMF is widespread but on large scale in agronomic, it is still not used in sufficient amount.

Keeping in mind the poor uptake and availability of P in soil for plants, the present study was undertaken to evaluate the combined effect of AMF inoculation, P level and planting method on growth and P uptake of maize for sustainable high crop production.

## Materials and Methods

**Experimental location:** Experiments were performed at the Research Farm of Agronomy department of The University of Agriculture Peshawar-Pakistan ( $34^{\circ}1'24''$  N and  $71^{\circ}28'18''$  E, 359 meters above sea level) during the year 2016 and 2017, with semi-arid subtropical climatic conditions with mean annual rainfall of *ca.* 450 mm. Data of the actual total monthly rainfall, relative humidity and minimum/maximum temperature of the experimental months from June to October during 2016 and 2017 are summarized in Figs. 1 & 2. However, irrigation was done when required. The farm had well drained silt loamy soil. Its physical and chemical characteristics (0-30 cm depth), in the years 2016 and 2017 were determined before sowing (Table 1).

**Table 1. Physical and chemical characteristics of soil (0cm-30cm depth) of the experimental field before sowing during the years 2016 and 2017.**

Properties	Unit	2016	2017
Soil texture	-	Silt loam	Silt loam
Clay particles	%	9.2	9.3
Silt particles	%	58	58.7
Sand particles	%	32.8	32
Soil pH	-	7.80	7.80
Electrical conductivity	$\text{d S m}^{-1}$	1.18	1.17
Organic matter	%	0.45	0.52
Total nitrogen	%	0.06	0.07
Phosphorus	$\text{mg kg}^{-1}$	2.8	3.5
Potassium	$\text{mg kg}^{-1}$	119.4	120.3
Mineral nitrogen	$\text{mg kg}^{-1}$	35.2	36.5

**Experimental design:** Each experiment consisting of three factors i.e. planting methods (PM), phosphorus (P) levels and arbuscular mycorrhizal fungi (AMF) application, was performed in RCB design (randomized complete block design) with split plot arrangement, four replications in each treatment. Main plots received PM and AMF treatments while subplots received various P levels. The plot size was  $4.5 \text{ m} \times 3.5 \text{ m}$ , with 6 rows 3.5 meter long. Row-row and plant to plant distance was kept 75 and 20 cm, for maize using open pollinated cultivar "Azam", respectively. The experimental field was continuously irrigated approximately for three weeks prior to planting for weeds, emergence and ploughed afterwards with cultivator and Rotavator. Seeds were sown on July 02 during 2016 and July 04 during 2017. PM included i. flatbed method, ii. raised bed method, and iii. Ridge planting method; AMF included i. AMF applied, and ii. AMF not-applied; and (c) P levels included 0, 30, 60, 90 and 120 kg Phosphorus/ha. AMF and P levels were applied before planting. Ridges and raised beds were made manually using spade in plots where sowing was carried out on ridges or on raised beds, respectively. Nitrogen and potassium were applied at the recommended rate of 120 and 60 kg/ha respectively. Nitrogen was applied in split dose; half at planting and the remaining half with 2<sup>nd</sup> irrigation when plants were at V3 to V4 stage. All potassium was applied at the time of planting. Weeding was done manually four to five days after the 2<sup>nd</sup> irrigation. Controlled flood irrigation was applied as and when required so that ridges and raised beds were not submerged in irrigation water.

**Soil analysis:** Before start of the experiment, soil samples were taken at five positions chosen at random in each subplot from 0-30 cm (depth) and analyzed for soil physical and chemical characteristics i.e. pH, EC, organic carbon and soil total nitrogen (N). Mineral nitrogen, extractable P and K were determined in composite samples. N, P and K minerals were determined in fresh soil samples. Whereas the remaining parts of the samples were dried at room temperature and grinded before using for soil total N, organic matter, pH and EC. For Soil textural classes' determination, the composite sample was sieved with a 2 mm sieve to remove any derbies of plants, pebbles or other unnecessary materials. The clay, sand and silt percentage of the soil sample was calculated with the help of the USDA textural triangle. Soil results are shown in Table 1 along with physicochemical analysis.

**Data collection:** Among the data, plant stature (PS) was calculated by a measuring tape from the bottom to tassel tip of each plant at silking stage. For this purpose, 5 plants of various statures in each plot were randomly selected in central four rows and their statures were measured with measuring tape. Statures of all these plants were then averaged and reported as PS. Number of leaves was counted in each of the five plants selected for PS in each plot at silking stage. The number of leaves was averaged per plant in each plot. Leaf area index (LAI) was calculated by calculating leaf area (Amanullah *et al.*, 2009) and then LAI determination by following the formula (Reddy, 2004).

LAI = Central two rows, plants leaf area ÷ Covered ground area

Specific leaf area (SLA) is a measure of the change in leaf area (LA) per unit of leaf dry weight (LW). It shows change in leaf thickness. SLA declines as the crop increase in dry weight and leaf area remains relatively constant (Reddy, 2004).

$$\text{SLA} = [(L_{A_1} \div LW_1) + (L_{A_2} \div LW_2)] \div 2 \text{ (Gardner et al., 1985)}$$

where:  $L_{A_1}$  = Leaf area at tasseling  $L_{A_2}$  = Leaf area at physiological maturity

Crop growth rate (CGR) is the increase in dry matter weight of a community of plants per unit area per unit time and calculated following formula (Gardner et al., 1985).

$$\text{CGR} = (W_2 - W_1) \div (T_2 - T_1) \div \text{GA},$$

where:  $W_1$  = Initial weight, and  $W_2$  = Final weight, GA = Ground area,

$T_1$  and  $T_2$  are the time internal (in days) at which data of  $W_1$  and  $W_2$  were collected respectively.

CGR was calculated tasselling and the stage of physiological maturity in the outer rows in each plot. Samples taken were oven dried for 48 hours and expressed in  $\text{g m}^{-2} \text{ day}^{-1}$ . Absolute growth rate (AGR) is defined as an increase in dry matter of a plant per unit time, calculated by using the formula by Gardner et al., (1985):

$$\text{AGR} = (W_2 - W_1) \div (T_2 - T_1)$$

where:  $W_1$  = Initial weight and  $W_2$  = Final weight,  $T_1$  and  $T_2$  is the time internal (in days) at which data of  $W_1$  and  $W_2$  were collected respectively.

AGR was calculated at tasseling stage and physiological maturity stage in the outer two rows in each subplot. The samples taken were oven dried for 48 hours and reported in  $\text{g day}^{-1}$ . SPAD Values were calculated with SPAD meter (Model TYS-A) by taking readings on leaves in five randomly selected plants (bearing ears) in each plot, to estimate the chlorophyll contents of leaf. Mean values of these readings were reported as SPAD value of each plot. For total P uptake calculation, first grain P uptake and stover P uptake were calculated. Grain P uptake was determined as described in Bovill et al., (2013):

$$\text{GPU} = \text{Grain P content (g / kg)} \times \text{grain yield (kg / ha)} \div 1000$$

Stover P uptake was determined as described in Bovill et al., (2013):

$$\text{SPU} = \text{Stover P content (g / kg)} \times \text{Stover yield (kg / ha)} \div 1000$$

Total P uptake was calculated as follow:

$$\text{Total P uptake} = \text{GPU} + \text{SPU}$$

**Statistical analysis:** ANOVA (analysis of variance) was used for RCBD data analysis by sing statistical software Statix-8.1. Means were compared using LSD test at 0.05 levels of probability, when the F-values were significant using the same mentioned software.

## Experimental Results

**Plant stature (PS):** Data relating to plant stature of maize as influenced by planting methods (PM), Phosphorus levels (P) and arbuscularmycorrhizal fungi (AMF) are given in Table 2. Statistical analysis of the data showed that P levels, PM and AMF had significant effect on plant stature of maize. The application of P fertilizer at different levels significantly affected the plant stature of maize. Taller plants (183 cm) were recorded at  $60 \text{ kg P ha}^{-1}$  which was not significantly dissimilar from stature of 181.2 and 181.4 cm obtained with 90 and 120  $\text{kg P ha}^{-1}$  respectively. Minimum plant stature of 173.7 cm was obtained with 30  $\text{kg P ha}^{-1}$ . Planting methods also affected plant stature and higher plants of 181.3 and 180.7 cm were produced in plots sown with raisedbed or ridge planting methods. Plots sown with flatbed method produced plant stature of 176.2 cm. AMF application had a positive impact on the plant stature by producing taller maize plants (182.3cm) as compared to non-AMF applied plots (176.5cm).

The interaction of PM x P had significant effect on plant stature of maize (Fig. 3). The figure showed that plant stature of maize was higher when sown with raisedbed followed with ridge planting method. The lowest plant stature was obtained when maize was sown with flatbed method. However, with raised bed method, higher plant stature was obtained with 60  $\text{kg P ha}^{-1}$  with decreasing trend with raise in P level to 120  $\text{kg ha}^{-1}$ . With ridge planting method, plant stature was at par at 60, 90 and 120  $\text{kg P ha}^{-1}$ , however, with flatbed method; plant stature was same with 30 and 120  $\text{kg P ha}^{-1}$ .

**Leaves per plant (L/P):** Data regarding number of L/P of maize as influenced by PM, P levels and AMF are given in Table 2. Data Analysis revealed that P levels, PM and AMF had significantly affected L/P of maize. Plants produced higher number of leaves (13.8 to 13.9) with P @ 60, 90 or 120  $\text{kg ha}^{-1}$ . Smaller number of leaves of 11.6 L/P were produced in plots where P was not applied. PM also affected number of L/P and higher number of 13.6 and 13.7 L/P were produced in crop sown with raisedbed or ridge PM. Plots sown with flatbed method produced on the average 12.6 L/P. The application of AMF also influenced L/P and more number of L/P (13.5) were observed with AMF application compared to plots not supplied with AMF (13 leaves). The interaction of PM x P regarding number of L/P is shown in Fig. 4. It clearly depicted that the PM had strong effect on the number of L/P at various P levels. Generally crop sown with flatbed method produced less number of leaves while the crop sown either with raisedbed or ridge method produced higher number of leaves. At 120  $\text{kg P}$ , L/P with flatbed method and raisedbed method were identical. The interaction P x AMF showed that the effect of P application at different rates in the presence of AMF had significant effect on L/P of maize (Fig. 5). Generally the plots applied with AMF produced higher number of L/P at all level of P except 30 and 60  $\text{kg P ha}^{-1}$  where the number of L/P were similar in plots which were provided with AMF or not.

**Leaf area index (LAI):** Data regarding LAI of maize as influenced by PM, P levels and AMF are given in Table 2. Analysis of the data showed that P levels, PM and AMF had significantly influenced LAI of maize. Plants having higher LAI (3.62, 3.64 and 3.72) were produced when these were

given P @ 60, 90 or 120 kg ha<sup>-1</sup>, respectively. Smaller LAI of 3.35 was produced in plots where P was not applied. PM also affected LAI and higher LAI of 4.65 and 4.42 were produced in crop sown with ridge planting or raisedbed methods respectively. Plots sown with flatbed method produced LAI of 3.52. The application of AMF also influenced LAI and higher LAI (4.55) was observed with AMF inoculation as compared to plots not inoculated with AMF (3.84). The interaction of PM x P regarding LAI of maize is shown in Fig. 6. It clearly depicted that PM had strong effect on LAI at various P levels. Generally, crop sown with flatbed method produced lower LAI while the crop sown either with raisedbed or ridge method produced higher LAI than that sown with flatbed method. There was gradual increase in LAI of the crop sown with flatbed method with increase in P level. However, the crop sown with raisedbed or ridge method recorded a sharp increase in LAI when P level was raised to 60 kg/ha. LAI was at par at 60, 90, and 120 kg P ha<sup>-1</sup>, sown with raisedbed or ridge method. The interaction P x AMF indicated that the effect of P application at different rates in the presence of AMF had significant effect on LAI of maize (Fig. 7). Generally the plots applied with AMF produced higher LAI at all level of P. However, at 60 kg P ha<sup>-1</sup> and above, LAI of plots applied with AMF was much higher than the plots without AMF.

**Specific leaf area (SLA):** Data regarding SLA of maize as influenced by PM, P levels and AMF are given in Table 2. Statistical analysis of data revealed that PM, Phosphorus

levels and AMF had significantly effected SLA of maize. Plants having higher SLA (309.6 and 307.9 cm<sup>2</sup> g<sup>-1</sup>) were produced when these were given P @ 90 or 120 kg ha<sup>-1</sup>, respectively. Lower SLA of 243 cm<sup>2</sup> g<sup>-1</sup> was produced in plots where P was not applied. PM also affected SLA and higher SLA of 298.8 cm<sup>2</sup> g<sup>-1</sup> was produced in crop sown with raisedbed method. Plots sown with flatbed method produced SLA of 269.5 cm<sup>2</sup> g<sup>-1</sup>. The application of AMF produced higher SLA (299.9 cm<sup>2</sup> g<sup>-1</sup>) as compared to plots not supplied with AMF (273.6 cm<sup>2</sup> g<sup>-1</sup>). The interaction of PM x P regarding SLA of maize is shown in Fig. 8. It showed that PM had significant effect on SLA at various P levels. Generally, crop sown with flatbed method produced lower SLA while the crop sown either with raisedbed or ridge method produced higher SLA than that sown with flatbed method. There was gradual increase in SLA of the crop sown with flatbed method with increase in P level till the level of 90 kg P ha<sup>-1</sup> beyond which the SLA stabilized. However, the crop sown with raisedbed or ridge method a sharp increase in SLA when P level was raised to 60 kg/ha. The SLA was at par with 60, 90, and 120 kg P ha<sup>-1</sup>, sown with raised bed or ridge method. The interaction P x AMF indicated that the effect of P application at different rates in the presence of AMF had significant effect on SLA of maize (Fig. 9). Generally the plots applied with AMF produced higher SLA at every P level. However, at 60 kg P ha<sup>-1</sup>, the SLA of AMF applied plots stabilized and showed a declining trend while the plots where AMF was not applied, SLA showed an increasing trend with increase in P level.

**Table 2. Plant stature (PS), leaves per plant (L/P), leaf area index (LAI) and specific leaf area (SLA) of maize as influenced by planting methods (PM), phosphorus levels (P) and arbuscular mycorrhizal fungi (AMF) averaged over two years 2016 and 2017.**

Treatments	PS (cm)	L/P	LAI	SLA (cm <sup>2</sup> /g)
P levels (kg ha <sup>-1</sup> )				
0	173.7 c	11.6 c	3.35 c	243.0 d
30	177.8 b	13.2 b	3.63 b	271.6 c
60	183.0 a	13.8 a	4.62 a	301.7 b
90	181.2 a	13.9 a	4.65 a	307.9 a
120	181.4 a	13.9 a	4.72 a	309.6 a
LSD (0.05)	2.5	0.3	0.16	3.6
Planting Methods				
Flatbed method	176.2 c	12.6 b	3.52 c	269.5 c
Raisedbed method	181.3 a	13.6 a	4.42 b	298.8 a
Ridge planting method	180.7 a	13.7 a	4.65 a	291.9 b
LSD (0.05)	2.1	0.2	0.14	3.3
AMF Application				
With AMF	182.3 a	13.5 a	4.55 a	299.9 a
Without AMF	176.5 b	13.0 b	3.84 b	273.6 b
Probability level	**	**	*	**
Years				
2016	178.9	13.1 b	4.17	285.8 b
2017	180.0	13.4 a	4.22	287.8 a
Probability level	Ns	*	Ns	*
Interactions				
PM x AMF	Ns	Ns	Ns	Ns
PM x P	**	**	**	**
AMF x P	Ns	**	**	**
PM x AMF x P	Ns	Ns	Ns	Ns

Means in the same category of rows or columns followed by at least one common letter(s) are not significantly different from each other at 5% level of probability

\*\* Means significant at 1%, \* means significant at 5% and Ns means Not-significant either at 1% or at 5% probability level

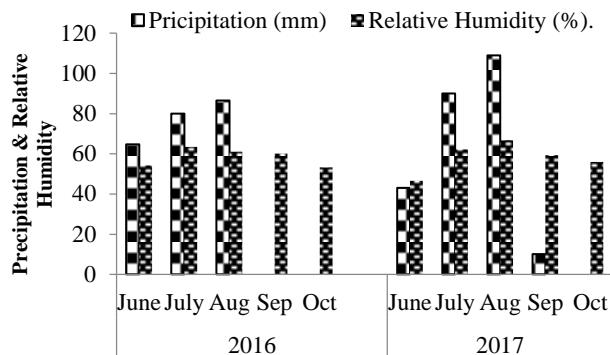


Fig. 1. Mean monthly precipitation (mm) and relative humidity (%) at the experimental site at the University of Agriculture Peshawar during 2016 and 2017.

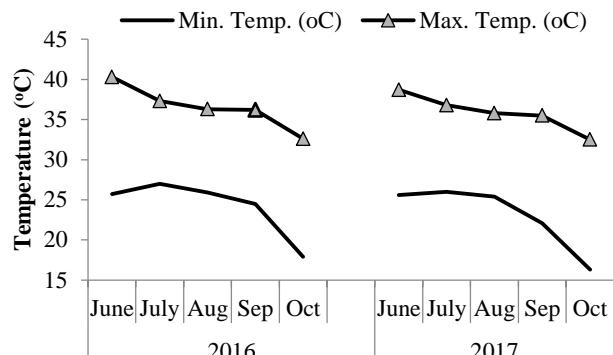


Fig. 2. Mean monthly minimum and maximum temperature (°C) at the experimental site (the University of Agriculture, Peshawar) during 2016 and 2017.

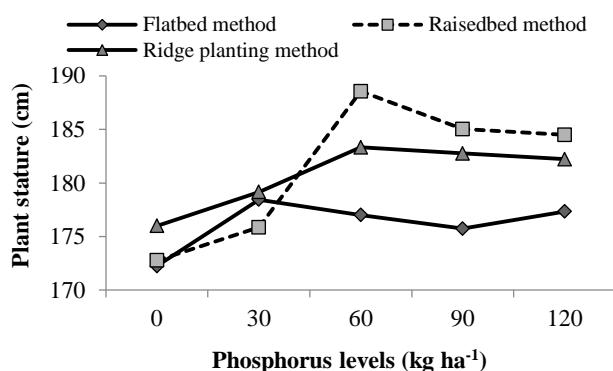


Fig. 3. Interaction of PM x P for plant stature (cm) of maize averaged over two years 2016 and 2017.

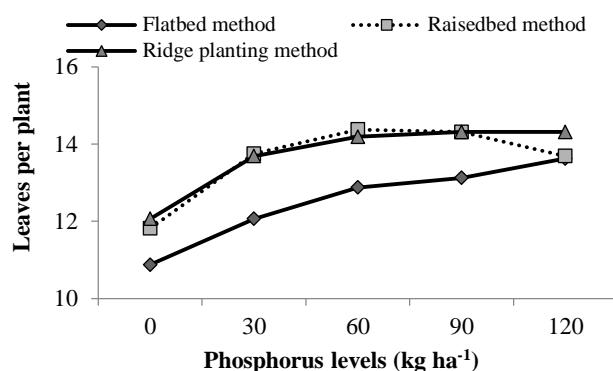


Fig. 4. Interaction of PM x P for number of leaves per plant of maize averaged over two years 2016 and 2017.

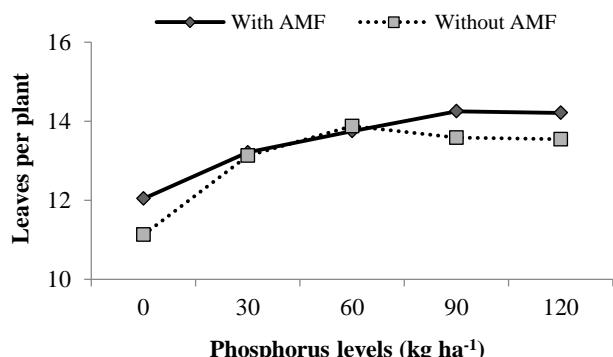


Fig. 5. Interaction of AMF x P for number of leaves per plant of maize averaged over two years 2016 and 2017.

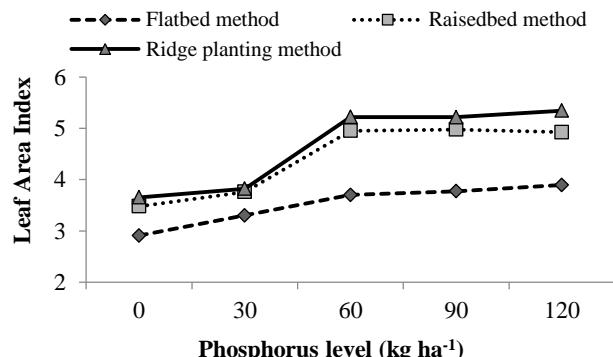


Fig. 6. Interaction of PM x P for leaf area index of maize averaged over two years 2016 and 2017.

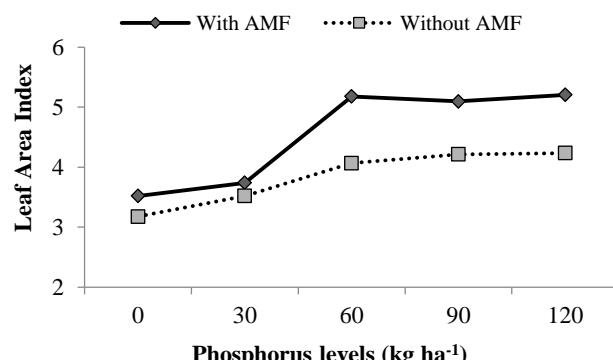


Fig. 7. Interaction of AMF x P for leaf area index of maize averaged over two years 2016 and 2017.

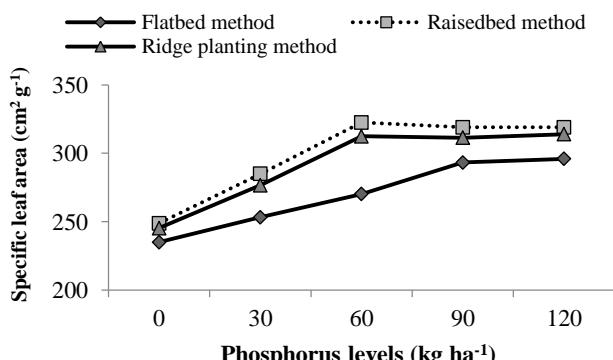


Fig. 8. Interaction of PM x P for specific leaf area of maize averaged over two years 2016 and 2017.

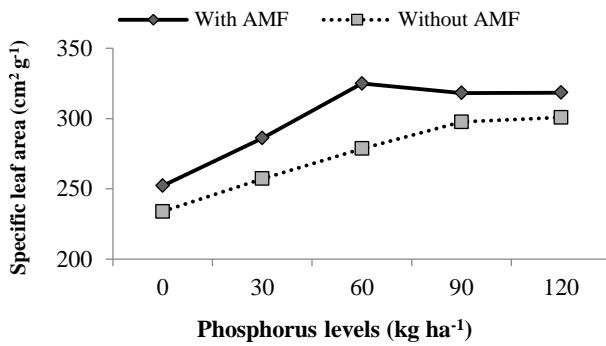


Fig. 9. Interaction of AMF x P for specific leaf area of maize averaged over two years 2016 and 2017.

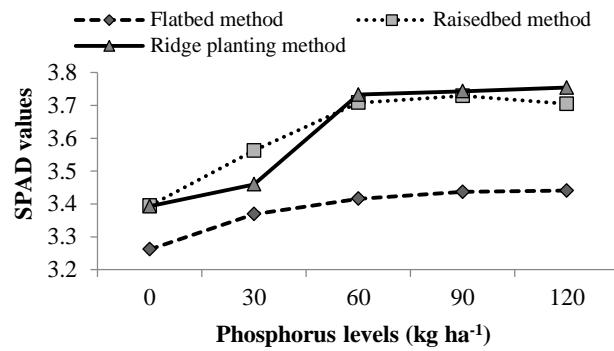


Fig. 10. Interaction of PM x P for SPAD values of maize averaged over two years 2016 and 2017.

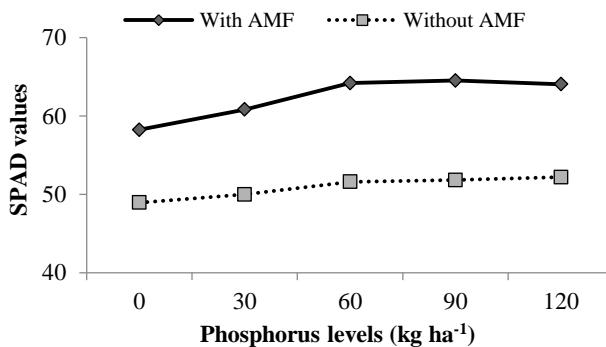


Fig. 11. Interaction of AMF x P for SPAD values of maize averaged over two years 2016 and 2017.

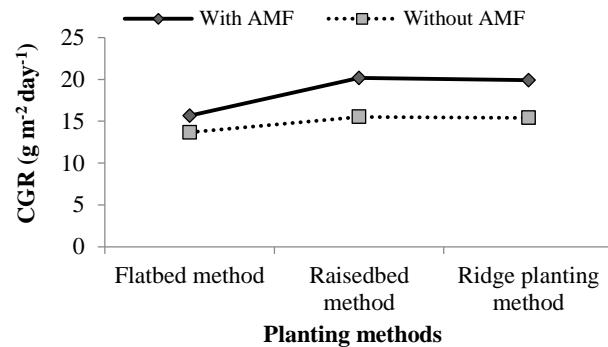


Fig. 12. Interaction of PM x AMF for crop growth rate ( $\text{g m}^{-2} \text{day}^{-2}$ ) of maize averaged over two growth stages (tasseing and maturity) and two years i.e. 2016 and 2017.

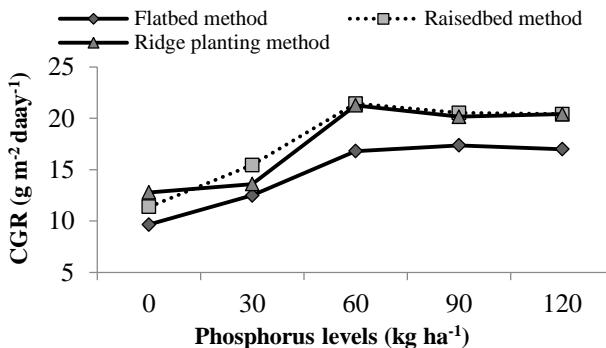


Fig. 13. Interaction of PM x P for crop growth rate ( $\text{g m}^{-2} \text{d}^{-1}$ ) of maize averaged over two growth stages (tasseing and maturity) and two years i.e. 2016 and 2017.

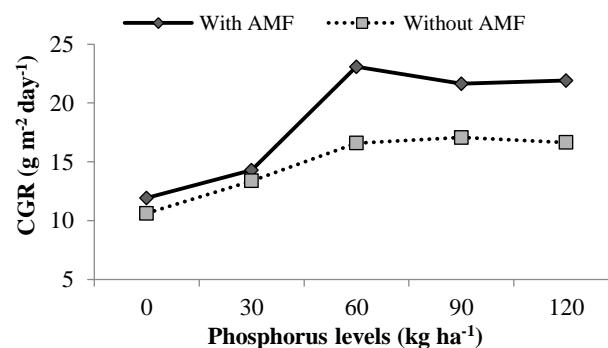


Fig. 14. Interaction of AMF x P for crop growth rate ( $\text{g m}^{-2} \text{day}^{-1}$ ) of maize averaged over two growth stages (tasseing and maturity) and two years i.e. 2016 and 2017.

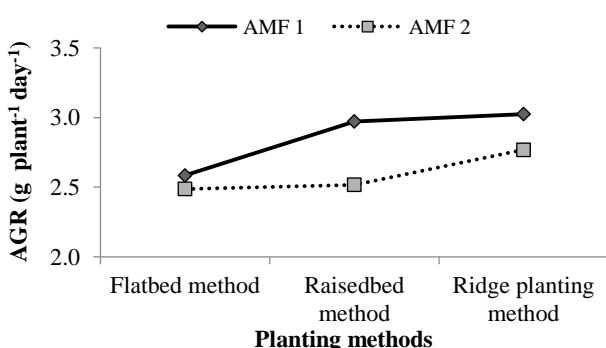


Fig. 15. Interaction of PM x AMF for absolute growth rate ( $\text{g plant}^{-1} \text{day}^{-1}$ ) of maize averaged over two growth stages (tasseing and maturity) and two years i.e. 2016 and 2017.

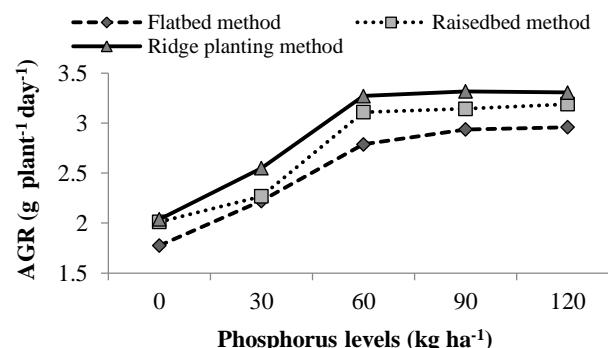


Fig. 16. Interaction of PM x P for absolute growth rate ( $\text{g plant}^{-1} \text{d}^{-1}$ ) of maize averaged over two growth stages (tasseing and maturity) and two years i.e. 2016 and 2017.

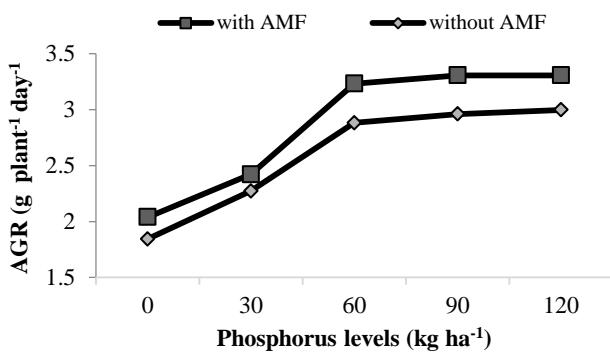


Fig. 17. Interaction of AMF x P for absolute growth rate ( $\text{g m}^{-2} \text{ day}^{-1}$ ) of maize averaged over two growth stages (tasseling and maturity) and two years i.e. 2016 and 2017.

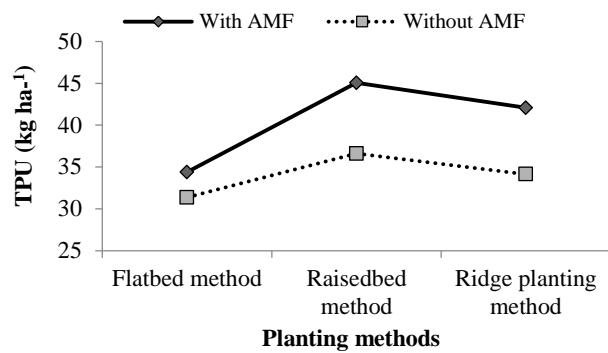


Fig. 18. Interaction of PM x AMF for total phosphorus uptake (TPU) ( $\text{kg ha}^{-1}$ ) of maize averaged over two years i.e. 2016 and 2017.

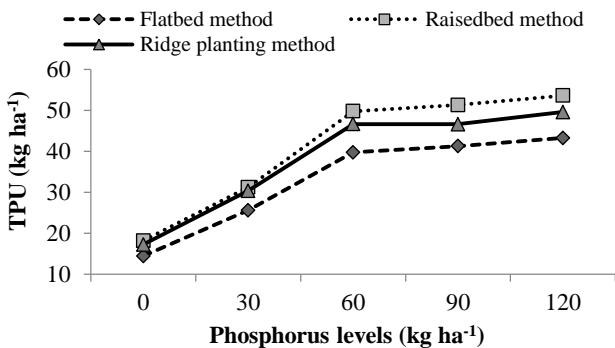


Fig. 19. Interaction of PM x P for total phosphorus uptake (TPU) ( $\text{kg ha}^{-1}$ ) of maize averaged over two years i.e. 2016 and 2017.

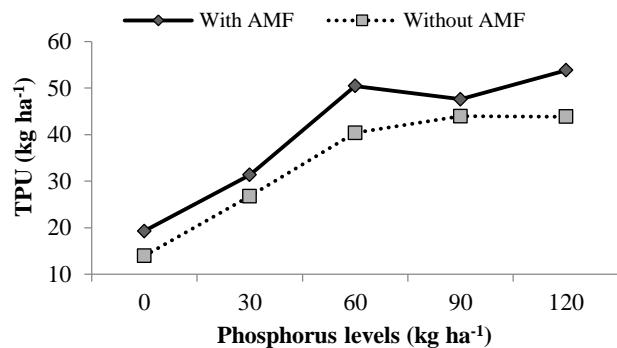


Fig. 20. Interaction of AMF x P for total phosphorus content (TPU) ( $\text{kg ha}^{-1}$ ) of maize averaged over two years i.e. 2016 and 2017.

Table 3. SPAD value, crop growth rate (CGR), absolute growth rate (AGR) and total phosphorus uptake (TPU) of maize as influenced by planting methods (PM), phosphorus levels (P) and arbuscular mycorrhizal fungi (AMF) averaged over two years 2016 and 2017. # Data of CGR and AGR are averaged over two growth stages i.e. tasseling and physiological maturity also.

Treatments	SPAD value	#CGR ( $\text{g m}^{-2} \text{ day}^{-1}$ )	#AGR ( $\text{g plant}^{-1} \text{ day}^{-1}$ )	TPU ( $\text{kg/ha}$ )
		P levels ( $\text{kg ha}^{-1}$ )		
0	53.6 c	11.3 d	1.94 c	16.6 d
30	55.4 b	13.8 c	2.35 b	29.1 c
60	57.9 a	19.8 a	3.06 a	45.4 b
90	58.2 a	19.4 ab	3.13 a	46.4 b
120	58.1 a	19.3 b	3.15 a	48.8 a
LSD (0.05)	0.8	0.41	0.32	1.4
Planting Methods				
Flatbed method	54.2 b	14.7 b	2.54 b	32.9 c
Raisedbed method	57.9 a	17.8 a	2.75 ab	40.8 a
Ridge planting method	57.9 a	17.6 a	2.90 a	38.1 b
LSD (0.05)	0.8	0.325	0.24	1.3
AMF Application				
With AMF	62.4 a	18.6 a	2.86 a	40.5 a
Without AMF	50.9 b	14.9 b	2.59 b	34.0 b
Probability level	**	**	**	**
Years				
2016	56.9	16.3 b	2.71	37.9
2017	56.4	17.1 a	2.74	36.7
Probability level	Ns	**	Ns	Ns
Interactions				
PM x AMF	Ns	**	**	**
PM x P	**	**	*	**
AMF x P	**	**	**	**
PM x AMF x P	Ns	Ns	Ns	Ns

Means in the same category of rows or columns followed by at least one common letter(s) are not significantly different from each other at 5% level of probability

\*\* Means significant at 1%, \* means significant at 5% and Ns means Not-significant either at 1% or at 5% probability level

**SPAD value:** Data regarding SPAD value of maize as influenced by PM, P levels and AMF are shown in Table 3. Statistical analysis of data revealed that P levels, PM and AMF had significantly affected the SPAD values of maize. Plants having higher SPAD values (58.1, 58.2 and 57.9) were produced when these were given P @ 120, 90 and 60 kg ha<sup>-1</sup>, respectively. These values were at par with each other. Smaller SPAD value of 53.6 was produced in plots where P was not applied. PM also affected SPAD value and higher SPAD value of 57.9 was produced in crop sown either with ridge planting or raisedbed method. Plots sown with flatbed method produced SPAD value of 54.2. The application of AMF produced higher SPAD value (62.4) in leaf as compared to plots not supplied with AMF (50.9). The interaction of PM x P regarding SPAD value of maize is shown in Fig. 10. The figure depicted that PM had strong effect on SPAD value of maize at various P levels. The crop sown with flatbed method produced lower SPAD value compared with the crop either sown with raisedbed or ridge method. There was gradual increase in SPAD of the crop sown with flatbed method with increase in P level. The crop sown with raisedbed produced higher SPAD value than the crop ridge method at 30 kg P ha<sup>-1</sup>. The SPAD value was at par at 60, 90, and 120 kg P/ha, either sown with raisedbed or ridge method. The interaction P x AMF indicated that the effect of P application at different rates in the presence of AMF had significant effect on SPAD value of maize (Fig. 11). Generally the plots applied with AMF produced higher SPAD value at all P levels. However, at 60 kg P ha<sup>-1</sup> and above, SPAD value of plants applied with AMF was much higher than without AMF.

**Crop growth rate (CGR):** Effect of AMF, PM and P levels on CGR of maize is shown in Table 3. The results show that AMF, PM and P levels affected the CGR of maize. Results regarding effect of Phosphorus application at different levels depicted that high value of CGR (19.8 g m<sup>-2</sup> day<sup>-1</sup>) was observed at 60 kg P ha<sup>-1</sup> which was at par with CGR (19.4 g m<sup>-2</sup> day<sup>-1</sup>) produced at 90 kg P ha<sup>-1</sup>. Lowest value of CGR (11.3 g m<sup>-2</sup> day<sup>-1</sup>) was noted at 0 kg P ha<sup>-1</sup>. Mean data for PM revealed the highest crop growth rate (17.8 g m<sup>-2</sup> day<sup>-1</sup>) for raised bed at par with ridge PM (17.6 g m<sup>-2</sup> day<sup>-1</sup>) and lower CGR (14.7 g m<sup>-2</sup> day<sup>-1</sup>) was noted in maize crop sown with flatbed method. Highest crop growth rate (18.6 g m<sup>-2</sup> day<sup>-1</sup>) was observed with AMF application, while lower CGR (14.9 g m<sup>-2</sup> day<sup>-1</sup>) was noted in plots without AMF application.

The effects of different PM in interaction with AMF (PM x AMF) on CGR of maize are clearly shown in Fig. 12. The figure showed that there was very distinct difference between AMF and non AMF applied plots in CGR of maize in which AMF applied plots recorded higher CGR in all PM. In flatbed method, the CGR in AMF applied plots was higher than in non AMF plots however, in raisedbed and ridge PM, the CGR in AMF applied plots was higher as compared with non-AMF applied plots. The effect of PM x P on CGR of maize shown in Fig. 13, depicted that there was very distinct difference among the PM in CGR of maize. Flatbed method recorded lower CGR at all P levels. It was

observed that in all PM, the CGR remained was at par with 60, 90 and 120 kg P/ ha and sufficiently higher than 0 or 30 kg P/ ha. The CGR in raisedbed and ridge methods was at par at 60, 90 and 120 kg P/ ha sufficiently higher than the CGR of maize in flatbed method at the mentioned levels of P. The effect of AMF x P on CGR of maize shown in Fig. 14, depicts difference between the AMF applied and non-AMF plots for CGR where the AMF applied plots recorded higher CGR at all P levels. It was observed that in non-AMF plots, the CGR gradually increased with increase in P level from 0 to 60 kg P/ ha, however, it remained the same with 60, 90 and 120 kg P /ha. In AMF applied plots, the CGR showed an abrupt increase when P level was increased from 30 to 60 kg P /ha. At 90 kg P, it showed moderate decrease and then showed stability at 120 kg P/ ha sufficiently higher than the CGR of maize recorded in non-AMF plots.

**Absolute growth rate (AGR):** Effect of AMF, PM and P levels on AGR of maize is shown in Table 3. The results depicted that AMF, PM and P levels affected the AGR of maize. Mean data for P levels revealed that higher AGR (3.152 g plant<sup>-1</sup> day<sup>-1</sup>) was noted at 120 kg P/ha which was at par with AGR of 3.133 and 3.056 g plant<sup>-1</sup> day<sup>-1</sup> produced at 90 and 60 kg P/ha. The lowest AGR (1.942 g plant<sup>-1</sup> day<sup>-1</sup>) was observed at 0 kg P/ha. Mean data for PM revealed the higher AGR (2.897 g plant<sup>-1</sup> day<sup>-1</sup>) for ridge planting which was at par with raisedbed method (2.745 g plant<sup>-1</sup> day<sup>-1</sup>) and lower AGR (2.536 g plant<sup>-1</sup> day<sup>-1</sup>) was noted in maize crop sown with flatbed method. In case of AMF application, higher AGR (2.861 g plant<sup>-1</sup> day<sup>-1</sup>) was observed with AMF application, while lower AGR (2.591 g plant<sup>-1</sup> day<sup>-1</sup>) was observed in plants grown without AMF application. The effect of PM x AMF on AGR of maize shown in Fig. 15, depicted that there was very distinct difference between AMF and non AMF applied plots for AGR of maize. AMF applied plots recorded higher AGR in all PM. In flatbed method, the AGR in AMF applied plots was higher than in non AMF plots, however, the difference was smaller. In raisedbed and in ridge methods the AGR in AMF applied plots was sufficiently higher as compared with non-AMF plots. The difference in effect of PM x P on AGR of maize is shown in Fig. 16. Flatbed method recorded lower AGR at all P levels. It was observed that in raisedbed and in ridge methods, the AGR remained the same with 60, 90 and 120 kg P/ha, and sufficiently higher than 0 or 30 kg P/ha. In flatbed method, the AGR was at par at 90 and 120 kg P/ ha sufficiently higher than the AGR of maize recorded at 0, 30 and 60 kg/ ha. The effect of AMF x P on AGR of maize shown in Fig. 17, depicted clear difference between the AMF applied and non-AMF plots for AGR of maize. The AMF applied plots recorded higher AGR at all P levels. It was observed that in non-AMF plots, the AGR gradually increased with increase in P level from 0 to 60 kg P ha<sup>-1</sup>, however, the AGR remained the same with 60, 90 and 120 kg P ha<sup>-1</sup>. In AMF applied plots, the AGR showed an abrupt increase when P level was increased from 30 to 60 kg P ha<sup>-1</sup>. At 60, 90 and 120 kg P, the AGR remained stable in the mentioned treatments.

**Total P uptake (TPU):** Data regarding TPU of maize as affected by PM, P levels and AMF are shown in Table 3. Statistical analysis of the data showed that P levels significantly affected TPU. Higher TPU ( $48.8 \text{ kg ha}^{-1}$ ) was produced with P application @  $120 \text{ kg ha}^{-1}$ . It was followed by at par TPU values of  $46.4$  and  $45.4 \text{ kg ha}^{-1}$  when the crop was applied P @  $90$  and  $60 \text{ kg ha}^{-1}$  respectively. Lower TPU of  $16.6 \text{ kg ha}^{-1}$  was produced in plots where P was not applied. PM also affected TPU and larger TPU was produced in crop sown with raisedbed method ( $40.8 \text{ kg ha}^{-1}$ ) followed by TPU of  $38.1$  and  $32.9 \text{ kg ha}^{-1}$  recorded by sowing with ridge or flatbed methods, respectively. In case of AMF application, higher TPU of  $40.5 \text{ kg ha}^{-1}$  was recorded with AMF application than TPU of plots not supplied with AMF ( $34 \text{ kg ha}^{-1}$ ). The effect of PM x AMF on TPU of maize shown in Fig. 18, showed that irrespective of the PM, plots where AMF was applied produced higher TPU as compared with the plots where AMF was not applied. In flatbed method, there was very little difference between the TPU of the plots applied with AMF or not. However, the TPU of the plots sown with raisedbed or ridge methods and applied with AMF were sufficiently higher than the plots where AMF was not applied. The effect of PM x P on TPU of maize shown in Fig. 19, revealed that there were some differences among the PM at various P levels for TPU of maize. The TPU recorded in flatbed method sown plots was the lowest among other the three methods at all P levels. A sharp increase in TPU was noted in flatbed and raisedbed method sown plots by increasing P level from  $0$  to  $60 \text{ kg ha}^{-1}$ . Above  $60 \text{ kg P}$ , the increase in TPU was mild. In plots sown with ridge method, TPU increased sharply with increase in P level was from  $0 - 60 \text{ kg}$ . Though, at  $90 \text{ kg}$  it showed a decrease and at  $120 \text{ kg P}$  again an increasing trend. The effect of AMF x P on TPU of maize shown in Fig. 20, revealed that the AMF applied plots recorded higher TPU at all P levels. TPU showed a sharp increase with increasing P level from  $0$  to  $60 \text{ kg P}$  in both with and without AMF plots. When P level was increased from  $60$  to  $120 \text{ kg}$ , TPU increased slowly only in plots without AMF. In plots of AMF, TPU increased sharply with increase in P level from  $0$  to  $60 \text{ kg}$ , but, at  $90 \text{ kg}$  it showed a decreasing and at  $120 \text{ kg P}$  again an increasing trend.

## Discussion

Plant stature is an index of growth and development representing the plant infrastructure build-up over a period of time. Though plant stature is genetically controlled but it may be modified by different agronomic practices. Data indicated that PM affected plant stature significantly. Raisedbed and ridge sown plants attained taller height than flatbed sown plants. Similar results on the effect of planting methods on plant stature were reported by Bakht *et al.*, (2006), Belachew & Abera (2010) and Tanveer *et al.*, (2014). Better soil physical conditions and moisture availability under bed and ridge planting might have helped the crop to maintain higher

growth rate as is evident from its stature. Application of different levels of P fertilizer significantly affected the plant stature of maize. Taller plants were produced in plots applied with P @  $60 \text{ kg ha}^{-1}$ , which were statistically at par with plant stature produced with P application @  $90$  and  $120 \text{ kg ha}^{-1}$ . Adequate application of P fertilizer is considered essential for rapid growth and improved quality of vegetative growth (Ayub *et al.*, 2002 and Masood *et al.*, 2011). P deficiency slows down the overall metabolic process and growth in plants (Rashid & Memon, 2001). Maqsood *et al.*, (2011), Ayub *et al.*, (2002) and Ibrahim and Kandil (2007) reported that increase in P levels had positive effect on maize height. AMF application significantly enhanced plant stature. As AMF augmented mineral absorption by host plant, especially low mobility mineral elements in the soil such as P, Zn and Cu through extensive exploration of the absorption surface and the volume of soil explored by fungal hyphae, it ultimately resulted in increased plant stature (Javot *et al.*, 2007). Koda *et al.*, (2018) reported that maximum height was recorded in the plants treated with AMF species. Laminou (2010) reported that AMF inoculation stimulated sorghum growth and ensured taller plants. Liu *et al.*, (2000) reported that root colonization by AMF resulted in enhanced uptake of relatively immobile metal micronutrients, such as Cu, Zn and Fe.

Leaves per plant also indicate plant growth and development. In this study, different PM significantly influenced the leaf count  $\text{plant}^{-1}$ . The leaf count  $\text{plant}^{-1}$  was higher in raisedbed and ridge sown maize than the flatbed sown. Higher leaf count  $\text{plant}^{-1}$  might be due to vigorous plant growth under raisedbed and ridge planting conditions. The reason for lesser value of leaf count  $\text{plant}^{-1}$  in flatbed planted crop might be the scanty water and nutrients availability compared to raisedbed and ridge sowing. Moisture availability in soil under raisedbed and ridge sowing is higher and for longer period compared to flatbed sowing (Singh, 2011).

Leaf area index (LAI) is defined as the ratio of leaf area (one sided) to a given unit of horizontal field soil surface area in a crop canopy. It is a dimensionless [ $\text{m}^2/\text{m}^2$ ] variable and a biophysical quantity. By definition, a crop having more total leaf area (determined by the single leaf area and the number of leaves) has more LAI. Leaf area is very important for crop's light interception and therefore has a strong influence on crop yield (Dwyer & Stewart, 1986). Leaf area index (LAI) is the indication of photosynthetic capacity of plant, markedly influencing the growth and yield of crop. Higher recorded value of LAI for raisedbed and ridge planting method at  $60 \text{ kg ha}^{-1}$  of P application, which were at par with the LAI values recorded at  $90$  and  $120 \text{ kg ha}^{-1}$ , and supported by Amanullah *et al.*, (2010). Similarly AMF inoculated plants produced more canopy due to larger root systems (Liu *et al.*, 2000; Koda *et al.*, 2018; Mathur *et al.*, 2018). The probable higher moisture content in soil profile of ridges and raisedbeds might have helped the plant to record better growth as compared to the flatbeds (Sing, (2011) and Tanveer *et al.*, (2014)). Reports published by Ortega *et al.*, (2008) and Bakht *et al.*, (2011) support our findings that maize planting through raised bed method is a proven efficient planting technique for cereal crops.

Higher SPAD value was recorded with moderate and higher P levels i.e. 60, 90 and 120 kg ha<sup>-1</sup>, which were significantly at par with each other. Similarly, raisedbed and ridge sown plants, and those treated with AMF affected SPAD value, positively. This could be due to the reason that AMF applied plants might have obtained nutrients in sufficient quantity which in turn assisted in synthesis of chlorophyll concentrations (Smith and Smith, 2011; Yan *et al.*, 2018) which ultimately resulted in improved SPAD value (Wang *et al.*, 2008; Mathur *et al.*, (2018). An important physiological trait of the plants is growth rate including crop growth rate and absolute growth rate. Growth rate is influenced by the inputs availability such as water and nutrient supply. In the present research findings, the overall growth rate was significantly enhanced, specifically in AMF inoculated plots under raised bed planting methods with moderate level of P application. The reason for increase in growth rate might be the nutrients availability under sufficient supply of P, AMF application and the better seed bed availability under ridge and raisedbed plantation (Lambers *et al.*, 2008; Bakht *et al.*, 2011).

Total P uptake was significantly improved with P levels applied @ 60 and 90 kg ha<sup>-1</sup>, in raisedbed and ridge methods and in AMF applied plots. Our results are supported with the studies of Sharif & Jan, (2008), Smith & Read, (2008), Cozzolino *et al.*, (2013) and Jan *et al.*, (2014) who reported that AMF enhanced the P uptake ability of crop plants from the soil. Wahid *et al.*, (2016), recommend AMF as a useful biofertilizers in combination with other microbes to improve the maize growth and total P uptake. Various studies have reported improvement in growth parameters of plants due to AMF inoculations which might be due to fine architecture of mycorrhizal hyphae, interacting with soil particles and facilitating the transport of non-mobile mineral elements particularly phosphorus to the plants (Liu *et al.*, 2000; Lambers *et al.*, 2008).

## Conclusion

Results obtained from the present study highlighted the beneficial role of AMF which improved the growth and P uptake of maize. It was concluded that application of AMF and P @ 60 kg/ha improved growth of maize. TPU is improved with AMF and P application @ 120 kg/ha. Among the planting methods, both raisedbed and ridge planting methods improved growth of maize. AMF application is recommended in P deficient calcareous soils of Pakistan. P fertilizer should be applied 60 kg/ha to the maize with AMF. Raisedbed & ridge planting methods for maize cultivation ensure better growth responses.

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