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Mycorrhizal Colonization in Different Varieties of Gladiolus and its Relation with Plant Vegetative and Reproductive Growth

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ABSTRACT

A field experiment was conducted in 2006-2007 to investigate the arbuscular mycorrhizal (AM) status of four gladiolus (*Gladiolus grandiflorus* L.) varieties viz. Oscar, Princesses Margaret Rose (PM Rose), Pricilla and Wind Song and its relationship with plant vegetative and reproductive growth. Mycorrhizal colonization status of the test gladiolus varieties was studied at three growth stages viz. early vegetative (2-3 leaf stage), late vegetative (5-7 leaf stage) and at flowering stage. In Oscar and PM Rose mycorrhizal colonization was gradually increased with the increase in plant age from early vegetative to flowering stage. By contrast, in Pricilla and Wind Song, there was a sharp and significant increase in mycorrhizal colonization from early to late vegetative growth stage and a decline thereafter at flowering stage. Data regarding various agronomic traits were recorded at flowering stage. All the vegetative and reproductive growth parameters except root length and biomass showed highly positive correlation with mycorrhizal colonization recorded at early vegetative growth stage.

Key Words: Arbuscular mycorrhizae; Gladiolus*;* Growth stages

INTRODUCTION

The term "Mycorrhiza" describes the symbiotic association between plant roots and certain soil fungi. Arbuscular mycorrhizal (AM) fungi are the most abundant in agricultural soils. These account for 5–50% of the biomass of soil microbes (Olsson *et al.,* 1999). About 80% of all terrestrial plants, including most agricultural, horticultural and hardwood crop species are able to establish this mutualistic association (Pozo & Azcón-Aguilar, 2007). AM fungi are ubiquitously associated with a great majority of plant families in different ecosystems across the world, ranging from the tropics (Zhao *et al.,* 2001) or arctic-alpine habitats (Haselwandter, 1987) to mesic (Muthukumar & Udaiyan, 2000) and arid habitats (O'Connor *et al.,* 2002). These fungi impart many benefits to plants. Colonization of roots by AM fungi has been shown to improve growth and productivity of several field crops (Javaid *et al*., 1994; Cavagnaro *et al.,* 2006; Pasqualini *et al.,* 2007) by increasing nutrient element uptake (Al-Karaki, 2006). These fungi are also known to enhance crop growth and yield through enhanced tolerance to various biotic (Khaosaad *et al.,* 2007) and abiotic stress factors (Al-Garni, 2006; Takeda *et al.,* 2007) and improving physical, chemical and biological properties of soil (Rillig & Mummey, 2006; Cardoso & Kuyper, 2006).

Gladiolus (*Gladiolus grandiflorus* L.) occupies a prominent position among the privileged cut flowers owing to the elegant appearance of its spikes of different hues and excellent vase life (Bose *et al.,* 2003). In the recent years, its demand and production in Punjab, Pakistan has increased tremendously owing to the showy flowering spikes. It is cultivated over 250 ha as commercial floral crop as well as potted plant in the commercial nurseries of Punjab Province (Anonymous, 2003). Recent studies show that gladiolus is a good mycorrhizal host (Javaid *et al*., 2007; Riaz *et al.*, 2007). Mycorrhizal fungi play an important role in increasing vase-life of cut flowers by reducing ethylene production (Besmer & Koide, 1999). Variation in mycorrhizal colonization in different varieties of the same species is well established (Rabbani *et al.*, 2002; Sensoy *et al.*, 2007). Keeping in view the importance of mycorrhizal colonization in flowering plants and variation in extent of mycorrhizal colonization and benefits among different genotypes of a plant species, the present research work was undertaken. The aim of this research work was to study the mycorrhizal status of different varieties of gladiolus growing under normal field conditions and its relationship with vegetative growth and flowering of the host plant.

MATERIALS AND METHODS

Soil characteristics. The soil of the experimental site was loamy textured having organic matter 0.7%, pH 7.8, available phosphorus 7 mg kg^{-1} and available potassium 100 mg kg^{-1}. The concentrations of micronutrients viz. boron,

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manganese, iron; copper and zinc were 1.06, 22.8, 10.8, 1.9 and 1.3 mg kg^{-1}, respectively.

Cultivation of gladiolus varieties. Experiment was conducted in field plots of 1.5×2 m. Bulbs of four gladiolus varieties namely Oscar, PM Rose, Pricilla and Wind Song, imported from Holland, were obtained from Sunny View Seed Store, Lahore. They were sown in rows with inter plant and inter row distance of 30 cm and 40 cm. There were 3 rows in each plot. Plants were irrigated with ground water whenever required.

Harvesting. Root of various gladiolus varieties were collected at early vegetative (2-3 leaf stage), late vegetative (5-7 leaf stage) and at full flowering stage. Ten replicate plants of each test variety were harvested. Data regarding root and shoot length and biomass, days to sprouting, days to flowering initiation, spike length and number of flowers per spike were recorded.

Mycorrhizal colonization study. Roots of the four test varieties collected at three growth stages were thoroughly washed under tap water and fine roots were cut into 1 cm pieces. The root samples were cleared and stained for AM study following Phillips and Hayman (1970). The roots were cleared for about 30 min. in 10% KOH solution in an autoclave, placed in 10% HCl for 10 min for neutralization and then stained with 0.05% glycerol-trypan blue solution.

Randomly selected, 30 stained root pieces of 1 cm each were studied for each sample. Root pieces were mounted in lactophenol on glass slides and studied under compound microscope. For percentage mycorrhizal colonization, each root piece was observed at 5 points under x 10 of the microscope and % mycorrhizal colonization was calculated. Arbuscular and vesicular colonization were quantified by counting these structures per 10 cm of root length.

Statistical analysis. Data regarding various plant growth and mycorrhizal colonization parameters were subjected to one way analysis of variance (ANOVA) followed by Duncan's Multiple Range Test (Steel & Torrie, 1980) to separate the means. Correlation between various plant vegetative and reproductive growth parameters (taken at flower stage) and mycorrhizal colonization parameters (taken at different growth stages) was computed using computer software Microsoft Excel.

RESULTS AND DISCUSSION

Vegetative growth. The bulbs of gladiolus var. Wind Song took maximum time i.e., 25 days to sprout followed by 19.3 by Oscar. However, the difference between these two varieties was insignificant. The other two varieties viz. PM Rose and Pricilla took 17.3 and 14.4 days, respectively to sprout. The sprouting time of these two varieties was significantly lower as compared to other two varieties (Fig. 1a).

Highest shoot length and biomass was recorded in PM Rose. Wind Song exhibited insignificantly lower shoot length and biomass as compared to PM Rose. In contrast to that the other two varieties namely Oscar and Pricilla showed significantly lower values of these two parameters as compared to PM Rose. The lowest values of shoot growth parameters were recorded in Pricilla those were significantly lower than the entire test gladiolus varieties (Fig. 1b & c).

Fig. 1. Comparison of different vegetative growth parameters of four test gladiolus varieties

Values with different letters show significant difference as determined by DMR Test.

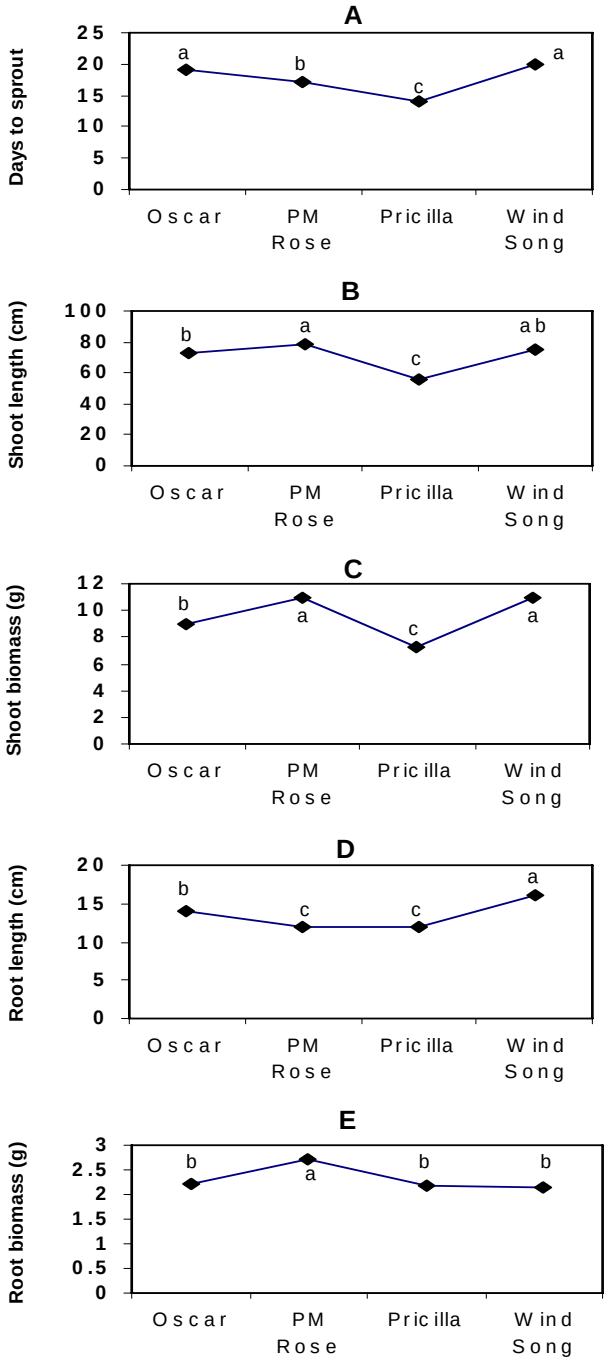


Table I. Correlation between mycorrhizal colonization at different growth stage with different plant vegetative and reproductive growth parameters of gladiolus at flowering stage

	Days to sprouting	Shoot length	Shoot biomass	Root length	Root biomass	Days to flowering	Spike length	No. of flowers/Spike
				Early vegetative growth stage				
MC	0.93	0.96*	0.88	0.64	0.21	0.95*	0.83	0.99**
NA	0.67	0.12	0.19	0.96*	-0.78	0.40	0.10	0.51
NV	0.57	0.27	0.53	0.82	-0.40	0.25	-0.07	0.59
				Late vegetative growth stage				
MC	0.17	0.01	0.37	0.50	-0.27	-0.16	-0.46	0.23
NA	0.48	0.37	0.67	0.66	-0.13	0.20	-0.09	0.57
NV	0.15	-0.13	0.21	0.55	-0.46	-0.20	-0.51	0.15
				Flowering stage				
MC	0.14	0.66	0.47	-0.38	0.88	0.44	0.66	0.31
NA	-0.62	-0.30	-0.53	-0.87	0.43	-0.31	0.01	-0.61
NV	0.00	0.44	0.70	-0.13	0.69	-0.04	-0.09	0.27

*, **, significant at P≤0.05, P≤0.01, respectively.
MC: Mycorrhizal colonization
NA: Number of arbuscules
NV: Number of vesicles

Similar to that of shoot biomass, highest root biomass was also produced by PM Rose that was significantly higher than all other test varieties. However, maximum root length was recorded in Wind Song that was significantly higher than rest of the test varieties (Fig. 1d & e).

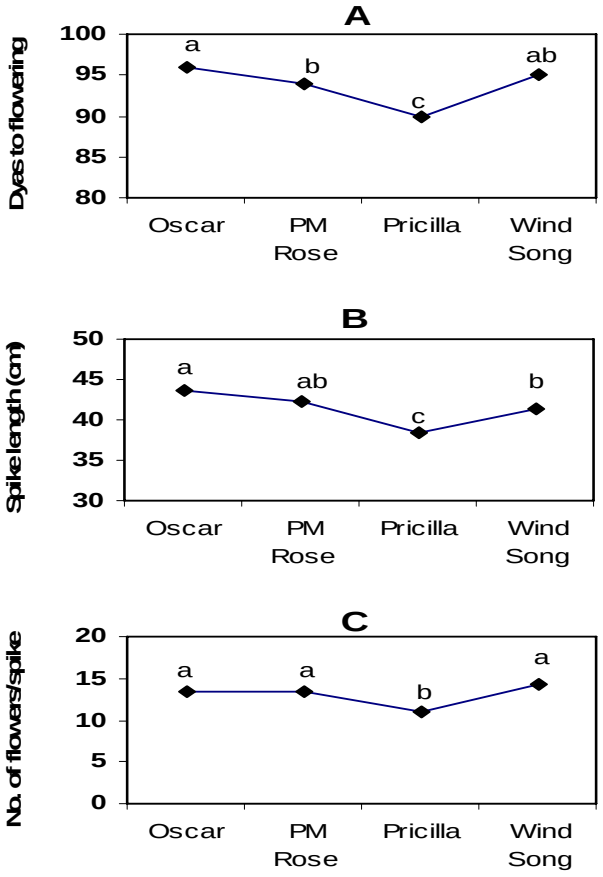
Reproductive growth. Gladiolus variety Oscar took maximum days i.e., 96 to initiate the flowering followed by 95 by Wind Song, 94 by PM Rose and 90 by Pricilla (Fig. 2a). Pricilla, the earliest flowering variety among the four test varieties, showed lowest spike length that was significantly different than spike lengths of all other test varieties. Number of flowers per spike was also lowest in this variety. Oscar was found best for having longest spike and Wind song for having maximum number of flowers per spike (Fig. 2b & c).

Mycorrhizal colonization. Oscar and PM Rose showed a similar pattern of mycorrhizal colonization at different growth stages. In both of these varieties mycorrhizal colonization was gradually increased with the increase in plant age from early vegetative to flowering stage (Fig. 3a). Recently Javaid *et al.* (2007) have reported a similar highest mycorrhizal colonization at flowering stage in another bulbous ornamental plant *Narcissus papyraceus* Ker-Gawl. Similarly, Pongrac *et al.* (2007) observed highest intensity of AM colonization in *Thlaspi praecox* in the flowering phase. By contrast, Pricilla and Wind Song exhibited a different mycorrhizal colonization pattern than the other two test varieties. In these varieties there was a sharp and significant increase in mycorrhizal colonization from early to late vegetative growth stage and a decline thereafter at flowering stage. In these varieties highest mycorrhizal colonization was recorded at late vegetative growth stage in contrast to that of Oscar and PM Rose where highest colonization was observed at flowering stage (Fig. 3a).

The most important function of arbuscular mycorrhizal fungi is thought to be the nutrient absorption from the soil to enhance the crop growth and yield (Smith & Read, 1997). The intraradical mycelium of the root cortex also extends from the root out into the soil where they

Fig. 2. Comparison of number of days to flowering initiation, spike length and number of flowers per spike in four test gladiolus varieties

Values with different letters show significant difference as determined by DMR Test



interface with soil particles. These extraradical hyphae function as absorptive structures for mineral elements and water (Bethlenfalvay & Linderman, 1992). The different mycorrhizal developmental pattern in various gladiolus varieties probably is due to different nutrient requirements

in the test varieties at different growth stages. The highest mycorrhizal colonization at late vegetative or at flowering stage in different varieties of gladiolus indicates that mycorrhizal colonization in this plant species plays an important role in meeting the enhanced nutrient requirements at these growth stages.

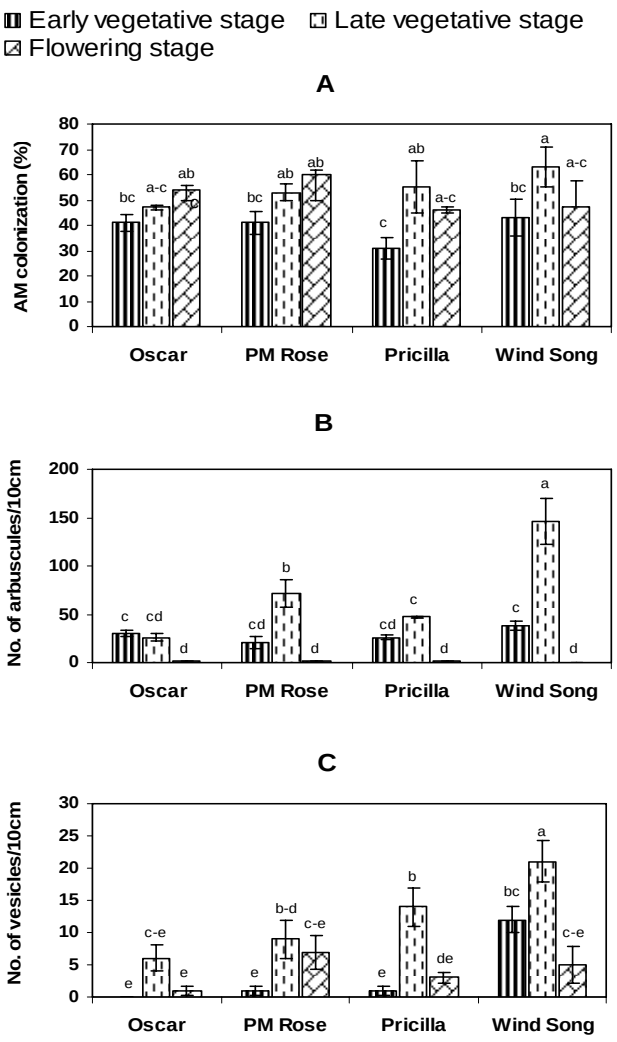
Although variation in number of arbuscules was recorded in different gladiolus varieties, however, arbuscular colonization pattern at different growth stages was very similar to one another in different varieties. At early vegetative growth stage, number of arbuscules was comparatively low. Highest number of arbuscules was recorded at late vegetative growth stage in all the varieties except Oscar. At flowering stage arbuscular colonization was negligible (Fig. 3b). Arbuscules are the structures where metabolites exchanges take place between the fungus and the host cytoplasm (Parniske, 2000). Arbuscules are short-lived. In most host-fungus interactions, they degenerate within 7 to 12 days (Gadkar *et al.*, 2001). In the present study highest arbuscular colonization at late vegetative growth stage reveals the possibility of maximum metabolic exchange between the symbiotic partners at this growth stage in the test varieties.

At early vegetative as well as at flowering stage, there was insignificant difference in number of arbuscules among the varieties. However, at late vegetative growth stage, a marked difference in number of arbuscules was recorded among the varieties. The highest number of arbuscules (146/10 cm) was recorded in Wind Song followed by PM Rose (72), Pricilla (47) and Oscar (26). The difference in arbuscular number among the varieties was significant for all the varieties except the difference between Oscar and Pricilla (Fig. 3b). Variation in mycorrhizal colonization among genotypes have also been demonstrated for marigold (Linderman & Davis, 2004), *Capsicum annuum* L. (Sensoy *et al.,* 2007) and tobacco (Janoušková *et al*., 2007). Regarding the genetic variation of marigold genotypes, or other plants reported in the literature for that matter, there must be variation in P requirements and the morphological and physiological capacity of the plant to acquire P independent of AM colonization. As discussed by (Parke & Kaeppler, 2000), P efficiency is reflected in the plant's ability to produce dry matter without the addition of P to the soil or growth medium in the absence of mycorrhizae.

Vesicular colonization pattern at various growth stages was very similar to one another in different test gladiolus varieties. Invariably all the test varieties exhibited an abrupt increase in number of vesicles from early to late vegetative growth stage and a decline thereafter at flowering stage (Fig. 3c). This typical pattern of vesicular colonization in various gladiolus varieties was different from generalized pattern of vesicular colonization in angiospermic plants. Usually vesicles are formed at later growth stages as terminal or intercalary swellings in the cortical cells and function as nutrient storage organs or as propagules in root fragments (Smith & Read, 1997).

Fig. 3. Comparison of different mycorrhizal parameters of four gladiolus varieties at different growth stages

Vertical bars show standard error of means of three replicates.
Values with different letters show significant difference as determined by DMR Test



Correlation between plant growth and mycorrhizal colonization. The correlation between various mycorrhizal and plants growth parameters is presented in Table I. All the vegetative and reproductive growth parameters except root length and biomass showed highly positive correlation with mycorrhizal colonization recorded at early vegetative growth stage. However, such correlations were lacking at later growth stages. Arbuscular colonization exhibited a significant and positive correlation with root length at early vegetative growth stage. The correlation between number of vesicles and various plant growth parameters was invariably insignificant at all the growth stages. The results of earlier studies regarding the correlation between plant growth and mycorrhizal colonization are contradictory. In a recent study, Javaid and Riaz (2008) reported a significant positive correlation of root and shoot biomasses with different

parameters of arbuscular and vesicular colonization in maize. Similarly, Xavier and Germida (2002) have reported a positive correlation between AM colonization of roots and total shoot dry matter production in lentil (*Lens culinaris* cv. Laird). By contrast, according to Al-Karaki and Clark (1998) enhanced plant growth may not always be related to degree of root mycorrhizal colonization in some plants.

CONCLUSION

The present study concludes that mycorrhizal developmental pattern in various gladiolus varieties is genotypic dependant. Furthermore, plant vegetative and reproductive growth in gladiolus is more related with mycorrhizal colonization at early growth stage as compared to colonization at later growth stages.

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