Indian J. Hort. 71(2), June 2014: 242-246

Utilization of native microbial isolates for sodic soil as commercial bio-regulators to increase yield and vase-life of gladiolus

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ABSTRACT

A study was conducted to determine the influence of endophytes along with other commercial nutrient mixtures on enhancing growth and improving vase-life of gladiolus. A field experiment was performed over the two successive years 2009 and 2010. The recommended dose of fertilizers was added and the treatment with regulators and their combinations were imposed during the critical stages of the crop. The commercial nutrient mixtures of Hi-Media Chemicals, India (HMN®), Biovita® and a bacterial strain CSR-B-1 from *Baccillus* isolated from sodic soils of Rae Bareli district, Uttar Pradesh was imposed as treatments and combinations. The results revealed that the bacterial strain CSR-B-1 alone and in combination with HMN significantly increased plant height, number of leaves/plant, spike length, number of cormlets, fresh and dry weight of cormlets and also the vase-life of the cut flower. The incremental effects on plants height, leaf number and leaf area were 32.32, 25.00 and 9.00%, respectively, while 13.00, 45.18 and 56.83% increment was noticed on number of plant, spike length and weight of spike, respectively as compared to control plants in both seasons. The role of bio-chemicals and enzyme like superoxide dismutase (SOD), phenyl alanine lyase (PAL) activities and phenols were also assessed and were attributed for increasing shelf-life and quality of the cut flowers.

Key words: Gladiolus, plant growth promoters, enzyme assay.

INTRODUCTION

Bulbs and corms belonging to the Liliaceae, Iridiaceae and Amaryllidaceae are major flower crops grown all over the world. Gladiolus belongs to Iridiaceae family and is available round the year for decorative use. It has originated in the Mediterranean areas of South Africa. The injudicious use of chemical fertilizers in agriculture has started causing various environmental problems. Therefore, it was suggested to replace some of these chemical fertilizers, growth regulators and micronutrient mixtures with bioagents like *Psuedomonas* and *Trichoderma*. Application of *Psuedomonas* strains 84 to banana roots of tissue cultured plants at the deflasking stage significantly improved plant growth and reduced infection of *Fusarium oxysporum* f. sp. c*ubense* in the rhizome under greenhouse conditions (Kavino *et al*., 12). Among the growth regulators gibberellins and cytokinins (Raja Ram *et al.*, 16) have been found to increase spike length and vase-life in gladiolus. Auxin, ethylene, abscisic acid and jasmonic acid have been detected in *Cyanobacteria*. *Cyanobacteria*, is a well known source of phytohormones in rice tissue culture. Thus, in this study we tried to assess the effectiveness of bacterial strain isolated from sodic soil as growth promoter apart from the bio-control property.

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MATERIALS AND METHODS

This study was carried out in the farmer's field at Hydergarh block of Uttar Pradesh under the National Agriculture Innovative Project (NAIP) of ICAR during two successive seasons of 2009 and 2010. The bulbs of gladiolus var. Nova Lux were planted in the first week of September at 50 cm × 20 cm spacing. The experimental plot (1 m × 1.5 m) contained 15 corms where all agricultural practices were followed. Plants were sprayed twice at three leaf stage and six leaf stage (critical stages) with freshly prepared solutions of Biovita® (sea weed extract) along with Hi-Media Nutrient (HMN®) as individual treatment and in combination with *Bacillus* strain CSR-B-1 isolated from rhizosphere region of grasses grown in sodic soils and evaluated at Central Soil Salinity Research Station, RRS, Lucknow. During the flowering period of each season, plant height, number of leaves/plant, spike length, number of florets/spike, number of cormlets were recorded. The physiological weight loss (%) at third and fourth day after harvest of spike was also recorded. Peroxidase enzyme assay was carried out using the reaction mixture (3 ml) consisting of (0.25 %) v/v guaicol in 10 mM potassium phosphate buffer (pH 6.9) containing 10 mM hydrogen peroxide

(Hammerschmidt *et al*., 10). The phenyl alanine lyase (PAL) assay was carried out using 400 ml of 50 mM Tris HCl (pH 8.8) and 600 ml of 1 mM L-phenylalanine along with enzyme extract as reaction mixture (Ross and Sederoff, 17).

Enzyme extract was stored in a deep freezer (-20°C) until used for biochemical analysis. Phenol content of gladiolus leaves was estimated as per the procedure given by Zieslin and Ben-Zaken (19) and expressed as catechol equivalents µg/g of protein. The catalase activity (EC 1.11.1.6) was assayed spectrophotometrically and expressed in mmol/min/g of sample. Superoxide dismutase (SOD) activity (EC 1.15.1.1) was determined as described by Belid El-Moshaty *et al*. (5) and was expressed in units/g tissue. The total indoles were determined in the methanolic extract, using P-dimethyl amino benzaldhyde test "Erlic's reagent" (Larsen *et al*., 15). Total soluble phenols were estimated calorimetrically (AOAC, 1) by using Folin-Ciocalteu reagent. Nitrogen (N), phosporous (P) and potassium (K) were determined according to the method described by Cottenie *et al*. (7).

RESULTS AND DISCUSSION

Data presented in Table 1 shows that foliar application of bio-regulators increased the plant height, leaf number and leaf area of the gladiolus. However, application of HMN + CSR-B-1 (T_4) at the critical stage of the growth significantly increased the plant height (102.33 cm), leaf number/plant (8.33) and leaf area (233.66 cm^2) compared with other treatments in both the seasons. The incremental effects on plants height, leaf number and leaf area were 32.32, 25.00 and 9.00, respectively over the control. The positive effect of the above parameter was also found in other treatments and their interactions. The positive effect of endophyte on growth was through enhancing cell division and expansion by increase in endogenous production of auxin and gibberellins. The present data (Table 2) emphasized that application of bio-regulators like HMN + CSR-B-1 (T_4) mixture and CSR-B-1 (T_2) significantly increased the spike length, number of florets and spike weight (g). The highest values were obtained in plants with T_4. The incremental effect on number of plant, spike length and weight were 13.00, 45.18 and 56.83, respectively as compared to control plants in both seasons. Pre-soaking and foliar spray of kinetin 50 ppm + AM increased plant height, early emergence of spike, number of florets per spike and vase-life (Kumar and Gupta, 14). Application of 100 ppm kinetin had positive influence on panicle emergence, while panicle length and rachis length were better with 100 ppm NAA (Joshi *et al*., 11). The treatment (T_4) gave the highest number of cormlets (16.50) and was statistically on par with T_2 (14.33) alone (Table 2). Similarly, the fresh and dry weight of the cormlets was the highest in T_4 (26.0 and 2.55 g, respectively) and also on par with T_2. It is evident from Fig. 1 that the physiological weight loss (PWL) was lowest in the treatment T_2 followed by T_4 than others at 3rd and 4th day of the harvest. These results were in agreement with earlier findings (El-Fawakhry and El-Tayeb, 8), where, the foliar application of amino acids on chrysanthemum had led to an increase in reproductive parameters.

The data in Table 3 showed that foliar application of HMN + CSR-B-1 strain (T_4) was the best treatment followed by CSR-B-1 (T_2). These treatments significantly increased SOD activity [56.51 units/g tissue (T_4) and 58.70 units/g tissue (T_2)], phenols [672.00 µg g^{-1} (T_4) and 690 µg g^{-1}(T_2)] and PAL [2265.30 nmol $min^{-1}ml^{-1}$ (T_4) and 2067.33 nmol $min^{-1}ml^{-1}$ (T_2)] in both seasons. The increased SOD and phenol contents reduce the availability of free radicals reducing the early senescence of flowers. SOD appears generally to decrease during leaf senescence.

Table 1. Effect of bio-regulators and nutrient mixtures on vegetative and reproductive growth of gladiolus plants.

Treatment	Days to emergence	Plant height. (cm)	Leaf No.	Leaf area (cm^2)
T_1	78.33	82.33	6.33	234.66
T_2	76.00	84.00	7.66	252.66
T_3	79.66	85.66	7.33	247.33
T_4	78.00	102.33	8.33	233.66
T_5	79.66	91.33	6.66	229.33
T_6	79.66	85.00	6.66	239.00
T_7	79.33	77.66	7.33	234.33
T_8	82.66	77.33	6.66	213.33
CD at 5%	2.33	5.16	1.00	21.51

T_1 = HMN, T_2 = CSR-B-1, T_3 = Biovita®, T_4 = HMN + CSR-B-1, T_5 = HMN + Biovita®, T_6 = Biovita® + CSR-B-1, T_7 = HMN + Biovita® + CSR-B-1 and T_8 = control

Table 2. Production of gladiolus cormlets and florets as affected by bio-regulators.

Treatment	No. of florets	Spike wt. (g)	Spike length (cm)	No.of cormlets	Cormlets FW (g)	Cormlets DW (g)
T_1	13.33	61.66	75.66	13.00	22.0	2.00
T_2	16.00	83.33	84.00	14.33	25.50	2.50
T_3	15.00	85.00	78.33	11.00	16.15	1.50
T_4	19.00	115.00	96.33	16.50	26.00	2.55
T_5	13.00	72.66	84.00	12.20	19.50	1.75
T_6	14.00	83.33	78.33	10.75	16.00	1.40
T_7	12.66	80.00	67.66	9.50	15.65	1.70
T_8	14.00	73.33	66.33	8.65	14.58	1.25
CD at 5%	1.83	17.53	8.86	2.12	2.65	0.33

T_1 = HMN, T_2 = CSR-B-1, T_3 = Biovita®, T_4 = HMN + CSR-B-1, T_5 = HMN + Biovita®, T_6 = Biovita® + CSR-B-1, T_7 = HMN + Biovita® + CSR-B-1 and T_8 = control

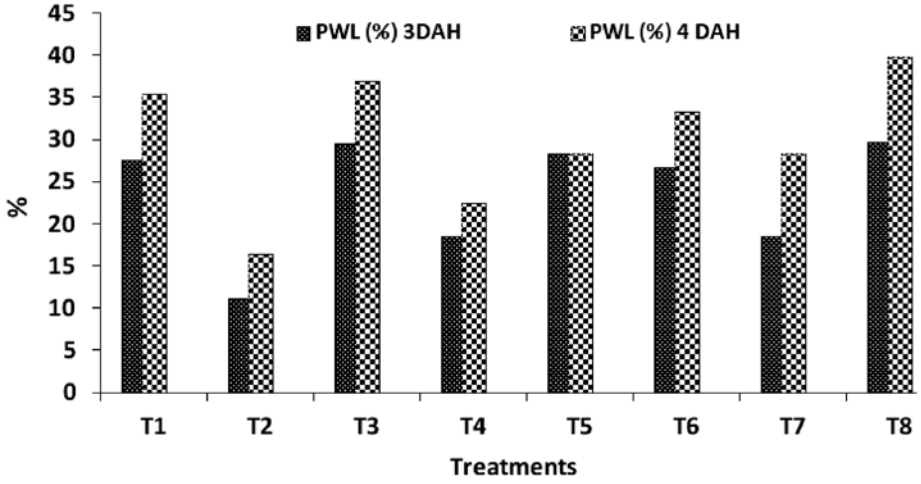

Fig. 1. Changes in physiological weight loss (PWL) percent after at 3rd and 4th days after harvest (DAH).

T_1 = HMN, T_2 = CSR-B-1, T_3 = Biovita®, Seaweed extract), T_4 = HMN + CSR-B-1, T_5 = HMN + Biovita®, T_6 = Biovita® + CSR-B-1, T_7 = HMN + Biovita® + CSR-B-1, T_8 = control.

The higher SOD activity may be associated with the inherent resistance mechanism of the flowers to the post harvest diseases (Anderson *et al*., 2). However, data further showed lower catalase and peroxidase activities in the treatment T_4 (HMN + CSR-B-1) and T_2 CSR-B-1 compared to T_5 (HMN + Biovita) and T_7 (HMN + Biovita® + CSR-B-1). It was evident that the bio-agent CSR-B-1 increased the shelf-life through increase in activities of SOD, PAL and phenols, while the activity of peroxidase and catalase was increased by the treatment with bio-agent Biovita®. The treatment with CSR-B-1 strain of *Bacillus* increased the activity of SOD, PAL and phenols which attributed for their delayed senescence in spite of their low peroxidase and catalase activities. Plants utilize specific enzymes to increase their defense mechanism towards the external stimuli (Banerjee *et al*., 4). Though T_8 (control) recorded comparatively higher catalase activity than T_2 and T_4, the reduced shelf-life with higher physiological weight loss was due to increased oxidation of available phenols, which was already present in lower amount (123.30 µg g^{-1}) compared to T_2 (690 µg g^{-1}). The higher phenols in T_4 and T_2 not only increased shelf-life but also inhibited from post-harvest diseases. Peroxidase is a multipurpose enzyme which catalyses the condemnation of phenols (Hammarschmidt *et al*., 10; Sivakumar *et al*., 18). The PAL and SOD activities also increased with the plants that exhibited higher shelf-life. The highest PAL activity was observed in T_4 (2265.30), which was also on par with T_2

Table 3. Changes in enzyme activity and biochemical contents of gladiolus at harvest.

Treatment	Peroxidase (abs $min^{-1}g^{-1}$)	Catalase (mmol/min/g)	PAL (nmol $min^{-1}ml^{-1}$)	Phenols (µg g^{-1})	SOD (units/g tissue)
T_1	0.85	22.50	1002.67	320.00	38.21
T_2	5.38	10.00	2067.33	690.66	58.70
T_3	3.7	21.50	1831.67	562.33	50.81
T_4	2.08	7.33	2265.30	672.00	56.51
T_5	6.66	43.00	1243.67	445.33	46.02
T_6	4.97	18.16	1623.33	553.00	52.01
T_7	7.56	15.66	1068.33	523.00	38.21
T_8	7.08	22.00	945.00	123.30	31.91
CD at 5%	0.30	5.06	82.61	15.12	1.83

T_1 = HMN, T_2 = CSR-B-1, T_3 = Biovita®, T_4 = HMN + CSR-B-1, T_5 = HMN + Biovita®, T_6 = Biovita® + CSR-B-1, T_7 = HMN + Biovita® + CSR-B-1 and T_8 = control

(2067.33) while the highest SOD was registered in T_2 (58.70) followed by T_4 (56.51). PAL is the first enzyme in the phenyl propanoid pathway and plays a key role in the synthesis of phenolic compounds in plants. These compounds are further converted to other phenolic compounds *via* coumarate such as flavonoids, anthocyanins and caffeic acid derivatives in plant tissues. The higher activity of PAL reduced the decay index of litchi fruit treated with chlorine dioxide (Bin Wu *et al.*, 6). The scavenging of oxygen by SOD results in the production of H_2O_2, which is removed by catalase (Asada, 3). Increase in the activities of the SOD, ascorbate peroxidase (APX) and glutathione reductase (GR) under salinity was also observed by various workers (Gueta-Dahan *et al*., 9).

With regard to the effect of CSR-B-1 and HMN on macronutrients (N,P and K), it was revealed (Table 4) that a significantly higher values in most cases was registered in T_2 and T_4 as compared with other treatments. Moreover, these two treatments were on par with each other. This was similar in the case of indoles also. The treatments with endophytes enhanced the uptake of nutrients through biological proceses. CSR-BIO a bio-product based on consortia of effective microbes facilitated the uptake of potassium, ferrous and calcium in bio-primed vegetables resulting in higher growth and yield. Accordingly, physiological performance of such plants was improved, as manifested by increased efficiency of roots in absorbing macro-nutrients from the soil.

It would be concluded that in the majority of the vegetative growth, flower quality and shelf-life of the treatment with HMN + CSR-B-1 (T_4) recorded the highest measurements followed by T_2 (CSR-B-1). The relationship with increased shelf-life and quality was also correlated with the biochemical changes in them.

Table 4. Effect of foliar application of bioregulators on indoles and macronutrients (%) of gladiolus plants.

Treatment	Indoles (mg/100 g FW)	Macronutrients (%)		
		N	P	K
T_1	32.25	1.21	0.22	0.27
T_2	40.35	1.62	0.24	0.38
T_3	35.60	1.25	0.20	0.32
T_4	45.65	1.65	0.24	0.37
T_5	34.50	1.20	0.20	0.25
T_6	36.30	1.28	0.17	0.30
T_7	38.25	1.40	0.22	0.35
T_8	27.20	0.55	0.10	0.20
CD at 5%	2.30	0.04	0.03	0.05

T_1 = HMN, T_2 = CSR-B-1, T_3 = Biovita®, T_4 = HMN + CSR-B-1, T_5 = HMN + Biovita®, T_6 = Biovita® + CSR-B-1, T_7 = HMN + Biovita® + CSR-B-1 and T_8 = control

Hence, it may be recommended that using HMN and CSR-B-1 could lead to beneficial results especially with respect to growth of the plant and flower quality in gladiolus.

ACKNOWLEDGMENTS

The authors thank National Agricultural Innovation Project, ICAR, New Delhi for financial assistance.

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Received: February, 2012; Revised: December, 2013; Accepted: January, 2014