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**Intergeneric grafting for enhanced growth,
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Intergeneric grafting for enhanced growth, yield and nutrient acquisition in greenhouse cucumber during winter

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Abstract

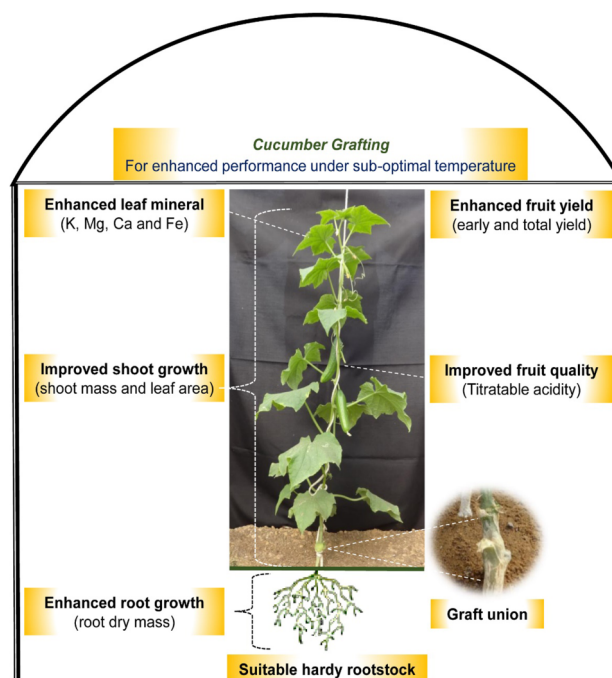
Aim : The objective of the present study was to evaluate the performance (based on growth, yield, quality, and nutrient acquisition) of commercial greenhouse cucumber (*Cucumis sativus*) grafted onto different locally available species as rootstocks during winter.

Methodology : The performance of cucumber cv. Infinity as scion was tested onto selected genotypes of three species of genus *Cucurbita* (pumpkin, squash and figleaf gourd), one each of *Lagenaria* (bottle gourd) and *Cucumis* (muskmelon) used as rootstocks. Plant growth, fruit yield and quality characteristics, and nutrient acquisition efficiency of rootstocks studied under prevailing sub-optimal temperatures during winter inside unheated greenhouse.

Results : The highest fruit yield was obtained in intergeneric cucumber grafting onto figleaf gourd followed by bottle gourd rootstocks, with increase in total yield of 30 and 10%, respectively over non-grafted cucumber. Fruit dry matter content in muskmelon grafted plants and titratable acidity in figleaf gourd and muskmelon grafted plants were also increased.

Interpretation : The improved performance of cucumber onto the cucurbit rootstocks, especially figleaf gourd was related to the increased root dry mass, root/shoot ratio and rootstock-stem thickness. This was also associated with the enhanced leaf nutrient status provided by vigorous root system of figleaf gourd rootstock under prevailing sub-optimal temperature.

Key words: Cucumber, Greenhouse, Sub-optimal temperature, Rootstock



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Introduction

Greenhouse cultivation is one of the fastest growing sectors of horticulture worldwide. In recent years, a great stride in its area expansion has been witnessed, particularly in the North Indian plains that extend up to North-western arid plains. Plastic covered greenhouse without artificial heating and cooling is commonly used protected structure in these areas. Here, cucumber is a predominant greenhouse crop because of its short growing cycle and growing demands by consumers for smooth-skinned seedless (parthenocarpic) cucumber. For grower's perspective, the off-season cucumber production under greenhouse is a lucrative, especially when the field grown cucumber fruits are not available during December to March. However, sub-optimal temperature prevailing under unheated greenhouses during winter decreases cucumber growth and production, especially when temperature falls below $\sim 18^{\circ}\text{C}$ (Papadopoulos, 1994), thus compelling the growers to compromise with relatively poor yield.

In some parts of the world, like in the European countries, artificial heating is an essential part of greenhouse management during winter, though it cannot be a viable solution as it involves huge cost of heating, and is not eco-friendly (Schwarz *et al.*, 2010). Use of low-temperature resistant cultivars can be an inevitable and sustainable option. However, the development of cultivars tolerant to abiotic stress has been a challenge owing to the genetic and physiological complexity of resistant traits (Schwarz *et al.*, 2010; Kumar *et al.*, 2017). A faster and efficient approach to enhance the efficiency of vegetable plants to adapt adverse environment by grafting on appropriate rootstock (Kumar *et al.*, 2017). In spite of its predominant use in controlling soil-borne pathogens (Rivard and Louws, 2008), grafting onto suitable rootstocks has shown promising results also in alleviating certain abiotic stresses such as salinity (Colla *et al.*, 2010), drought (Kumar *et al.*, 2017), heavy metals (Kumar *et al.*, 2015) and thermal stress in fruiting vegetables (Schwarz *et al.*, 2010; Ntatsi *et al.*, 2014; Li *et al.*, 2015 a,b).

In most cases, the major driving force in delivering the success of grafted plants under stressful conditions was rootstock's capability to maintain supply of water, nutrients, and certain metabolites that help in alleviating adverse effects of stress on scion shoots (Colla *et al.*, 2013; Kumar *et al.*, 2015; 2017). The damage caused by abiotic stresses such as low temperatures may vary with species, and the extent of changes in cellular components can be related to degree and magnitude of low temperatures exposure (Lee *et al.*, 2002). Further, different rootstocks may have different impressions and implications on shoot performance (Li *et al.*, 2015a). Depending on location and purpose different rootstock genotypes of the commonly used species like pumpkin, squash, figleaf gourd, bottle gourd etc. have been used for grafting cucumber in different studies (Colla *et al.*, 2013; Li *et al.*, 2015 a,b). Further, the utilization of locally available materials has been suggested to be an important strategy for any crop improvement programme targeted for

specific purpose and location (Kumar and Syamal, 2009; Pandey *et al.*, 2013). Exploitation of local genotypes as rootstocks is crucial, especially in countries, where grafting in vegetables is not so common, such as in India which possesses great diversity in many cucurbit species. In view of the above, the present study aimed at evaluating the performance (based on growth, yield, quality, and nutrient acquisition) of commercial greenhouse cucumber grafted onto different locally available species as rootstocks under sub-optimal temperature prevailing inside unheated greenhouse during winter in North-Indian arid plains.

Materials and Methods

Plant material and growth condition: The experiment was conducted in an unheated greenhouse (area 500 m^2) at Central Research Farm of ICAR-Central Arid Zone Research Institute, Jodhpur during winter season of 2015-16. The mean, maximum and minimum daily air temperature recorded in the greenhouse during the cropping period are presented in Fig.1. The relative humidity ranged from 65 to 85%. Cucumber cultivar 'Infinity' (Bayer Crop Science) was selected as scion. Five different cucurbit species used as rootstocks were figleaf gourd (*Cucurbita ficifolia*, a landrace from Ooty region), pumpkin (*C. moschata* cv. BSS 750), winter squash (*C. maxima* cv. Arka Suryamukhi), bottle gourd (*Lagenaria siceraria* cv. Sharda) and muskmelon (*Cucumis melo* cv. Kesar) with respective designation as FLG, PM, WS, BG and MM. The non-grafted cucumber was designated as Cc. Seeds of scion as well as rootstocks were sown in soilless medium (vermiculite: cocopeat; 1:2 ratio) during 1st week of November. Grafting was performed during last week of November following cleft grafting method (Lee *et al.*, 2010).

Grafted seedlings were placed in a growth chamber at 25°C and relative humidity of 90%. Plants were kept in dark for first 36 hrs and then the light was gradually increased with decreasing relative humidity to 80% over a period of 4-6 days. Plants were gradually shifted from partial light to full light inside the greenhouse. Transplanting was done at 2-3 true leaf stage (11th December) in paired rows at $50\text{ cm} \times 45\text{ cm}$ spacing on 90 cm wide beds. Plants were trained to single stem on the trellis by regular pinching of side shoots. The experiment was conducted in a randomized complete block design with three replications. Each experimental unit consisted of ten plants.

The greenhouse soil had pH 7.8, organic carbon (OC) 0.22%, total N 0.03%, available P 16.3 kg ha^{-1} and available K 221.5 kg ha^{-1} . The soil contained 85% sand, 8.1% silt and 5.5% clay, and classified as coarse-loamy, mixed, hyperthermic Camborthids according to US soil taxonomy. One week before transplanting, 25 t ha^{-1} compost was mixed by tilling in the top (15 cm) soil. At final soil working, the fertilizers (60:60:80 kg ha^{-1} , N:P:K) were mixed in the soil. During crop cycle, additional fertilizers of 60 kg N and 80 kg K along with 12 kg Ca and 4 kg Mg per hectare were given through fertigation during whole cropping period. In addition, once a week a commercial grade micronutrients (Aeris Agro Ltd. Mumbai) were applied through

drip. Similar plant protection measures to control pests and diseases were followed during the period of study.

Plant growth, fruit yield and quality parameters: At harvest, plants were separated into leaf, stem and root, and their dry weight were determined after oven-drying at 65°C until constant weight was obtained. Root to shoot ratio was calculated by dividing measured root dry mass by the sum of leaf and stem dry mass. At harvest, the stem girth of scion as well as rootstock above and below the graft union was measured with a digital caliper. The leaf area was electronically measured with Li3100c Area Meter (Li-Cor Bioscience).

The fruit yield (kg plant⁻¹) and fruit number were determined by combining the fruits obtained from each harvest. Mean fruit weight, fruit length and fruit girth were measured by averaging the fruits from three different harvests. Fruit quality analysis was conducted on freshly harvested fruits during peak harvesting period. Total soluble solids (TSS) was determined with a digital handheld refractometer (Bellingham and Stanley, UK). Potentiometric titration with 0.1 M NaOH up to pH 8.1 by using 10 ml of juice was employed as per Colla *et al.* (2013). The pH of fruit juice was measured with a pH meter (HI-2215; Hanna Instruments). Fruits dry matter content was determined by weighing the dried fruits kept in a forced air oven at 70°C until constant weight was obtained.

Leaf mineral analyses: Plant leaves were oven-dried at 65°C until constant weight was obtained. Dried leaf samples were then grounded in a Willy Mill. Diacid (HNO₃ + HClO₄ in 9:4 ratio) and analyzed for phosphorous by vanadomolybdo-phosphoric acid yellow colour method as outlined by Jackson (1973). Potassium was determined by flame photometry as described by Chapman

and Pratt (1961) whereas calcium and magnesium were determined using versene titration method (Derderian, 1961). Sulphur was estimated by colorimetric method using barium chromate (Palaskar *et al.*, 1981). The concentration of total micronutrients *viz.*, zinc, iron and copper in diacid digested samples was determined by Atomic Absorption Spectrophotometer (GBC 932AA).

Statistical analyses: Data were statistically analyzed by analysis of variance using SPSS software package (SPSS version 22 for Windows, 2013). Duncan's Multiple Range Test was performed (P = 0.05) to separate the treatment means within each variable measured.

Results and Discussion

Cucumber is a thermosensitive crop and temperature below 18°C may diminish its growth and production (Papadopoulos, 1994; Li *et al.*, 2015a). In the present study, cucumber plants (both grafted and non-grafted) experienced sub-optimal temperatures for a considerable period, especially during early growth stage (Fig. 1). The significant differences for various growth parameters such as shoot (stem + leaf) and root dry mass, root/shoot ratio, rootstock-stem girth and leaf area among different cucumber graft combinations clearly indicate differential sensitivity of rootstocks to sub-optimal temperatures (Table 1). Among the tested rootstocks species, figleaf gourd was found superior by displaying better growth promotive response to cucumber shoots. Plants grafted onto figleaf gourd rootstock produced highest root dry mass, rootstock-stem girth, leaf dry mass and leaf area. Besides, bottle gourd rootstock also displayed a slightly better response on cucumber performance than control. However, the record of lowest values for most of the

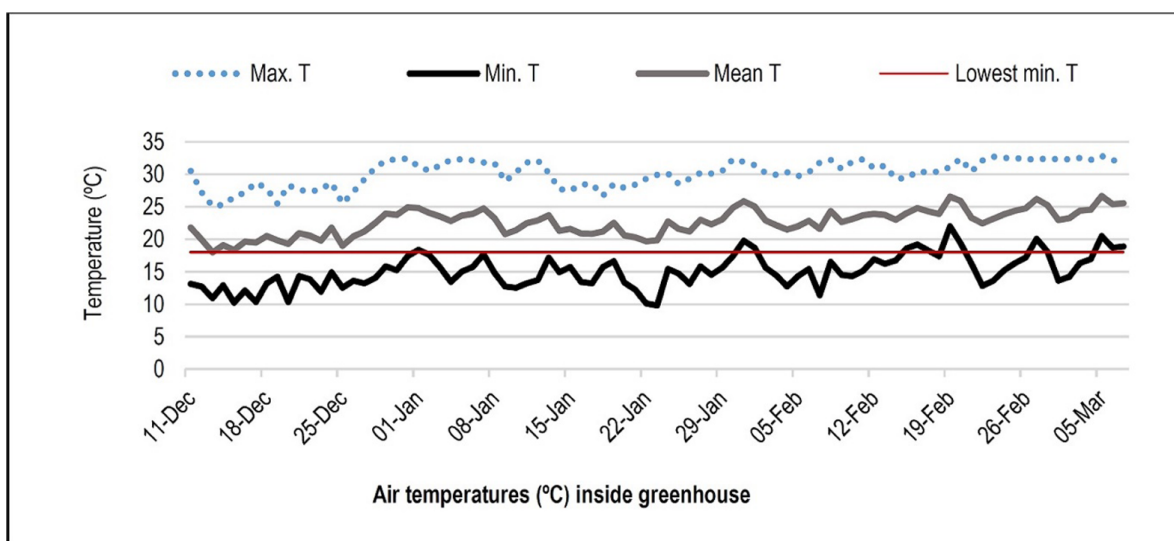


Fig. 1: Mean, maximum, minimum and required lowest minimum air temperatures during cropping period (December to March) inside greenhouse.

Table 1: Effect of graft combination on plant growth parameters of greenhouse cucumber during winter

Graft combination	Dry mass weight (g plant ⁻¹)			R/S	Stem girth (mm)		Leaf area (m ²)
	Leaf	Stem	Root		Rootstock	Scion	
Cc	76.99 ^b	16.79 ^a	6.40 ^{bc}	0.069 ^a	8.32 ^c	8.32	1.76 ^{bc}
Cc/MM	61.06 ^c	11.46 ^b	5.60 ^c	0.077 ^b	7.99 ^d	8.21	1.49 ^d
Cc/PM	79.06 ^{ab}	16.50 ^a	6.58 ^{ab}	0.069 ^a	9.43 ^b	8.28	1.71 ^c
Cc/WS	83.03 ^a	17.03 ^a	6.73 ^{ab}	0.069 ^a	9.87 ^b	8.43	1.87 ^b
Cc/BG	89.22 ^a	16.82 ^a	7.35 ^a	0.069 ^a	9.26 ^b	8.53	1.85 ^b
Cc/FLG	100.15 ^a	18.03 ^a	7.23 ^{ab}	0.061 ^a	12.03 ^a	8.61	2.06 ^a
Significance	**	***	***	**	***	NS	***

Mean values of three replicates within columns are separated using Duncan's multiple range test $P = 0.05$; NS, **, ***, non-significant or significant at $P < 0.01$ or 0.001 , respectively

growth parameters in muskmelon rootstock-grafted plants indicated high sensitivity of muskmelon rootstock to sub-optimal temperature. Next to muskmelon rootstock grafted plants, the sensitivity to sub-optimal temperature was likely highest in non-grafted cucumber plants (control) as evident by similar root dry mass, rootstock-stem girth and leaf area (Table 1). It is evident from the measured growth parameters that figleaf gourd rootstock, by virtue of its vigorous root system as reflected by high root mass and rootstock-stem girth, provides more vigour to cucumber shoots under prevailing sub-optimal temperature condition. Li *et al.* (2015a) also observed growth promotive response of figleaf gourd rootstock on cucumber shoots under low-temperature. Furthermore, leaf area in figleaf gourd grafted plants was 12% higher than non-grafted plants which indicated diversion of more assimilates produced by greater leaf area that has contributed higher fruit growth, and finally the fruit yield. The contribution of leaf area in improved shoot physiology, growth and yield has been also demonstrated in grafted vegetable plants under stressful environments (Colla *et al.*, 2013; Kumar *et al.*, 2015).

Rootstock genotypes significantly influenced yield and yield-related attributes (Table 2). Fruit yield ranged from 2.43 to

4.30 kg plant⁻¹ in different graft combinations. Cucumber grafted onto figleaf gourd rootstock showed 30% higher yield than non-grafted plants. Yield increase was also recorded in cucumber plants grafted onto bottle gourd (10%) and winter squash (3%), however the increase in latter was non-significant. Since, fruit yield is influenced by certain yield attributing characteristics such as number of fruits, fruit size (weight and girth) and shape (Kumar *et al.*, 2015), cucumber fruit yield in the present study also corresponded well to increase of many of these yield attributing characters. Compared to control, the increase in average fruit number, mean fruit weight and fruit girth in figleaf gourd rootstock grafted plants was 17.5, 12.6 and 9%, respectively.

The findings of the present study are in agreement with those reported by Colla *et al.* (2013) for mean fruit weight and Velkov and Pevicharova (2016) for fruit number and fruit weight and their contribution to yield increase in cucumber. Unlike grafting effects onto *Cucurbita* and *Lagenaria* rootstocks, cucumber grafting onto muskmelon rootstocks showed depression in fruit yield by 19.6%, characterized by a reduction in mean fruit weight (15%), a decrease in fruit length (17.9%) and fruit girth (2.3%), compared with control (Table 2). It is likely that

Table 2: Effect of graft combination on fruit yield and yield related parameters of greenhouse cucumber during winter

Graft combination	Fruit yield (kg)	Fruit no.	Mean fruit weight	Fruit length	Fruit girth
	(plant ⁻¹)		(g)	(cm)	
Cc	3.02 ^{bc}	15.21 ^b	215.4 ^b	18.83 ^a	3.83 ^b
Cc/MM	2.43 ^d	14.47 ^b	184.3 ^c	17.18 ^b	3.56 ^c
Cc/PM	2.92 ^c	14.30 ^b	218.6 ^b	18.67 ^a	3.77 ^{bc}
Cc/WS	3.11 ^{bc}	14.93 ^b	223.5 ^b	18.77 ^a	3.91 ^b
Cc/BG	3.31 ^{bc}	15.41 ^b	230.6 ^{ab}	19.06 ^a	3.84 ^b
Cc/FLG	4.30 ^a	17.85 ^a	242.6 ^a	19.2 ^a	4.19 ^a
Significance	***	***	***	**	***

Mean values of three replicates within columns are separated using Duncan's multiple range test $P = 0.05$; Significance **, *** at $P < 0.01$ or 0.001 , respectively

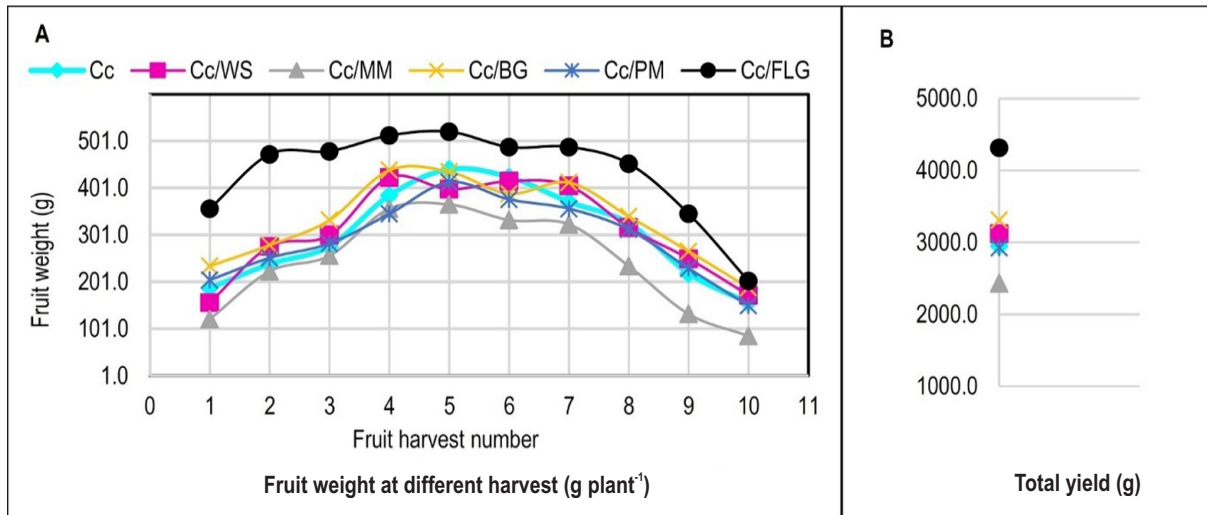


Fig. 2 : Per plant fresh fruit weight (g) of cucumber A) at different harvest, and B) sum of all the harvests (i.e., total fruit weight) in different graft combination of greenhouse cucumber during winter.

there was some incompatible reaction between scion and rootstock that caused restriction in water and nutrient flow from graft union (Davis *et al.*, 2008). The data of fresh fruit weight at different harvest dates indicated that fig leaf gourd rootstock in spite of providing highest total fruit yield per plant also resulted in the early fruit harvest (Fig. 2). Compared to control as well as other graft combinations, shoot growth was vigorous in figleaf gourd rootstock grafted plants that have likely resulted in earlier flowering and fruiting. Due to the fact that figleaf gourd originates from highlands in South America and widely grown in cooler regions even at high elevation of South-East Asia, including India (Lim, 2012), its high adaptability to sub-optimal temperature condition is expected. Previous reports also highlight the capability of figleaf gourd tolerance to sub-optimal temperature than cucumber, besides good tolerance to low light (Li *et al.*, 2015a,b), thus its suitability to winter season.

There was no obvious effect of grafting on determined fruit quality characteristics, except those grafted onto muskmelon rootstock in which increase in fruit dry matter content was observed (Table 3). However, a perceptible increase in titratable acidity (TA) was noted, especially on figleaf gourd rootstocks grafted plants. The increase in fruit TA was also reported by previous workers when *Cucurbita* species were used as rootstocks for grafting cucumber (Colla *et al.*, 2013). The higher fruit dry matter content in muskmelon rootstock grafted plants might be due to poor performance of muskmelon roots under sub-optimal temperature stress for water availability together with essential nutrients (Table 3). Variable growth and yield response of cucumber plants may partly be ascribed to differential ability of root systems for uptake and/ or translocation of nutrient elements under sub-optimal temperature. Among the rootstocks, figleaf gourd was able to enhance accumulation of certain nutrient

Table 3: Effect of graft combination on fruit quality characteristics of greenhouse cucumber during winter

Graft combination	Dry matter (%)	TSS (°Brix)	pH	Titratable acidity (%)
Cc	3.41 ^b	3.93	5.51	0.11 ^{ab}
Cc/MM	3.55 ^a	4.01	5.46	0.12 ^a
Cc/PM	3.36 ^b	3.96	5.45	0.10 ^b
Cc/WS	3.39 ^b	4.00	5.49	0.11 ^{ab}
Cc/BG	3.43 ^b	3.98	5.45	0.11 ^{ab}
Cc/FLG	3.39 ^b	3.91	5.59	0.12 ^a
Significance	**	NS	NS	**

Mean values of three replicates within columns are separated using Duncan's multiple range test P = 0.05; NS, **, non-significant or significant at P < 0.01, respectively

Table 4: Effect of graft combination on leaf nutrient concentration of greenhouse cucumber during winter

Graft combination	P	K	Ca	Mg	S	Zn	Fe	Cu
	(g kg ⁻¹ dry weight)					(mg kg ⁻¹ dry weight)		
Cc	2.18	48.7 ^{bc}	27.7 ^a	3.88 ^{bcd}	5.18	10.28	34.1 ^b	2.75
Cc/MM	1.88	43.3 ^c	21.2 ^b	2.75 ^d	5.15	8.88	23.8 ^c	2.58
Cc/PM	2.13	48.0 ^{bc}	26.1 ^a	3.45 ^{cd}	4.75	9.65	33.6 ^b	2.80
Cc/WS	2.40	50.8 ^{ab}	27.1 ^a	4.23 ^{abc}	4.86	9.73	34.5 ^b	2.79
Cc/BG	2.30	51.9 ^{ab}	27.8 ^a	4.75 ^{ab}	5.40	10.05	38.8 ^{ab}	2.26
Cc/FLG	2.18	54.2 ^a	29.8 ^a	5.34 ^a	4.93	10.56	43.5 ^a	2.59
Significance	NS	**	***	***	NS	NS	***	NS

Mean values of three replicates within columns are separated using Duncan's multiple range test $P = 0.05$; NS, **, ***, non-significant or significant at $P < 0.01$, <0.001 , respectively

elements (i.e., K, Ca, Mg and Fe) in leaves of grafted cucumber as compared to non-grafted plants (Table 3). On the other hand, concentrations of P, S, Zn and Cu did not vary significantly among non-grafted and grafted plants onto other rootstocks, except muskmelon rootstocks (Table 3). Graft combination involving muskmelon rootstock invariably accumulated the lowest amount of nutrients in their leaves as obvious from poor root growth characters (low root dry mass, rootstock-stem thickness). This shows rootstock related varying response of grafting in nutrient accumulation. Grafting had no effect on N and P contents, while it increased the content of K in the shoots of grafted cucumber compared to non-grafted plants (Canizares *et al.*, 2005). However, Huang *et al.* (2010) did not find any change in the concentration of N, P and K in cucumber leaves caused by rootstock under abiotic stress.

The differences in results for a particular nutrient in these two cases can be due to the difference in growth conditions and rootstock genotypes. In the present study, P content remained same, while K content differed greatly in response to the use of rootstocks, the highest recorded in figleaf gourd, while lowest in muskmelon rootstock grafted plants. Wang *et al.* (2013) reported the response of K in cold tolerance crop plants. Higher K tissue concentrations can reduce the sub-optimal temperature-induced damage that is possibly due to K led induction of antioxidants system and accumulation of some metabolites, increased photosynthesis, stomatal movement and osmoregulation (Marschner, 2012; Wang *et al.*, 2013). Likewise, higher leaf concentration of K caused a favorable effect on the growth and production of figleaf gourd rootstock grafted plants in similar fashion. Micronutrients content such as Mg and Fe in leaves was high in plants grafted onto figleaf gourd that was likely found to maintain the physio-biochemical processes of plants more active under stressful conditions. These two nutrients are known to contribute biosynthesis of photosynthetic apparatus either by involving in structural components such as in leaf chlorophyll or acting as a cofactor of enzymes (Marschner, 2012). The present results on micronutrients are in accordance with the results of Huang *et al.* (2010), who reported an appreciable increase in Fe

content, while Zn and Cu content remained same in leaves of grafted cucumber onto vigorous rootstock under abiotic stress.

In conclusion, grafting onto suitable rootstock (figleaf gourd) better adapted to low-temperature represents an effective tool to successfully grow low-temperature sensitive cucumber inside unheated greenhouse during winter in North-Indian plains, and in the areas where temperature remains below the optimum range in temperate regions.

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