

Micropropagation of vegetable type aloe vera (*Aloe barbadensis* Mill.)

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Abstract

Micropropagation protocol for vegetable type Aloe vera (*Aloe barbadensis* Mill.) has been developed using shoot tip explants. The protocol standardized for multiple micro-sucker proliferation, induction of *in vitro* adventitious roots and acclimatization of rooted micro-sucker from *in vitro* environment to greenhouse condition. Maximum proliferation of micro-suckers was obtained with combined supplementation of Benzyl adenine (BA) 2 mg + Thidiazuran (TDZ) 0.5 mg + 0.1 mg Naphthalene acetic acid (NAA) to per liter basal Murashige & Skoog (MS) medium containing 0.8% agar and 3 % sucrose. The maximum rootable micro-sucker (~2 m) long were achieved after transfer of culture to hormone free medium for two weeks. The elongated micro-suckers were rooted successfully under *in vitro* condition. The better quality adventitious roots formation observed with NAA 0.5 mg/l supplemented to ½ MS medium. The regenerated plants were successfully acclimatized under Hi- Tech greenhouse and maintained in protrays for further growth and development

Keywords.: Aloe vera, micropropagation, micro-suckers.

Introduction

Aloe vera (*Aloe barbadensis* Mill.) is an important plant which belongs to family Liliaceae and extensively used in therapeutic uses (Cera *et al.*, 1980 ; Davis and Leiter, 1988, Hart *et al.*, 1998 ; Anshoo *et al.*, 2005). Recently, it is widely used in several cosmetic preparations and culinary purpose particularly in many districts of Rajasthan. The leaf pads and flower stalks are mainly used for culinary and pickle preparation. Among several genotypes only sweet type Aloe vera is used for culinary preparation (Saroj *et al.*, 2004).

Aloe vera can be grown in arid and semi-arid regions as ground story crop in orchards or agro-forestry models. Its gel is very effective for treatment of wounds, burns and other skin disorders, placing a protective coat over the affected area, speeding up the rate of healing and reducing the risk of infection. Quality Aloe vera pads produce can be harvested when grown under partial shade conditions.

Its commercial cultivation for vegetable farming requires large quantity of planting material of sweet type genotype which cannot be obtained through vegetative sucker production since the *in vivo* sucker production in Aloe vera is rather slow and insufficient to meet the demand for commercial vegetable farming. *In vitro* propagation offer a possibility to improve the regeneration efficiency of Aloe vera. Several attempts have been made for *in vitro* propagation of Aloe vera with varied success with different combination of plant growth regulators (Meyer and Staden, 1991., Aggarwal and Barna 2004, Fattachi *et al.*, 2004, Wenping *et al.*, 2004). The hormonal requirements for *in vitro* regeneration are varied for different genotypes (Ray and Sarkar 1991, Chaudhuri and Mukundan, 2001., Liao *et al.*, 2004, Wenping *et al.*, 2004, Saroha *et al.*, 2005). Under present investigation regeneration efficiency of sweet type genotype of Aloe vera was evaluated with different combination of plant growth regulators using shoot tip explants.

Materials and Methods

Preparation of mother stock plants

The mother plant of Aloe vera was planted in greenhouse at a spacing of 60 x 60 cm in well prepared

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beds. The suckers of 10-12 cm height were collected from the mother plants and washed thoroughly in running tap water for excision of shoot tip explant.

Preparation and sterilization of explant

The expanded leaves of sucker and basal portion below 2.0 cm to the shoot tip were washed with distill water containing 0.1% Tween-20 for about 10-15 minutes. The explants rinsed with water and soaked for 30 minutes in 0.2% Bavistin solution. The shoot tip after through rinsing with distill water, were then subjected for surface sterilization with 3-4 minutes under laminar flow hood. Explants were dissected and trimmed to the size (~ 1.0 cm) for inoculation onto different medium for micro-sucker proliferation.

Culture media and conditions for microsucker proliferation

Surface sterilized shoot tip explants were inoculated on MS medium supplemented with 30 g sucrose, 8.0 g agar and various concentration of BA (1, 2, and 4 mg/l) alone or in combination of TDZ 0.5 mg/l and NAA 0.1 mg/l. The pH of the medium was adjusted to 5.7 and sterilized by autoclaving at 121°C 15 lbs for 15 minutes. The details of the proliferation medium are given in Table 1. The cultures were incubated at 26±2°C, 16 hrs photoperiod provided by cool fluroscent tube with 1000.lux intensity. Observation with respect to multiple sucker production were recorded after I and III subculture. The period of each subculture was four weeks.

Culture medium for rooting

After passing proliferated cultures onto elongation phase for two weeks (i.e. MS medium without hormone)

the elongated micro-suckers of about 2 cm long were used for rooting experiment. The micro-sucker transferred to rooting media containing ½ MS medium supplemented by 3.0% of sucrose, 0.8% agar and varied levels of auxin such as IAA and NAA (0.1, 0.5, 1.0 and 2.0 mg/l).

Acclimatization of plantlets

For hardening of the plantlets in arid ecosystem, a three step hardening procedure was adopted by transferring rooted micro-suckers into protrays containing potting mixtures of vermiculite and cocopeat/vermicompost in the ratio of (3:1) and the plantlets were kept under acclimatization hood (25±2°C temperature and 80-90% RH). 5000 lux intensity for 10 days. Thereafter, transferred to green house with 28±5°C temperature 60-70% RH) under 150000 lux light intensity. The acclimatized plantlet were further transferred to environmental controlled glass-house covered with shade net of 50% light intensity for further growth and development. Statistical analysis: The observations recorded with respect to different parameters were analysed by calculating the mean value of 10-15 replicates (cultures) in each treatments. The experiment were arranged in completely randomized design for statistical analysis.

Results and discussion

Surface sterilization of Aloe vera is extremely important because the suckers normally emerged from underground stem and axillary buds, which host a large variety of microorganism. In this experiment, the expanded leaves of sucker and basal portion below 2.0 cm to the shoot tip was removed and wash with distill water containing 0.1% Tween-20 for about 10-15 minutes was important. The treatment with mercuric chloride was not sufficient for *in vitro* establishment of culture. Thus, the

Table 1. Effect of BA and NAA on the percentage of responsive explants and multiple sucker production

	Treatments MS + PGR (mg/l)			% of responsive explants	No. of microsuckers explants after 4 weeks of inoculation	No. of microsuckers explants after third sub culture	Average length of microshoots (cm)
	BA + TDZ + NAA						
T ₁	0.0	+0.0	+0.0	0	1.0	1	1.7
T ₂	1.0	+0.0	+0.0	100	2.0	3.4	2.2
T ₃	2.0	+0.0	+0.0	100	2.0	4.0	1.9
T ₄	4.0	+0.0	+0.0	60	2.0	3.2	1.6
T ₅	1.0	+0.5	+0.0	100	2.2	4.5	1.9
T ₆	2.0	+0.5	+0.0	100	2.0	3.2	1.6
T ₇	4.0	+0.5	+0.0	60	2.0	2.6	1.3
T ₈	1.0	+0.5	+0.1	100	3.2	8.0	2.3
T ₉	2.0	+0.5	+0.1	100	3.6	12	1.9
T ₁₀	4.0	+0.5	+0.1	60	2.0	4	1.7
CD					0.17	0.71	0.30

Table 2. Effect of different auxins and their concentration on the root formation in microsucker

Auxin	Concentration mg/l	% of microsucker with roots	No. of root/microsucker	Average length roots (cm.)
Control	0.0	40	1.8	1.2
IAA	0.1	45	1.9	1.5
	0.5	50	2.0	2.1
	1.0	50	2.1	1.5
	2.0	60	2.5	1.9
	0.1	100	3.0	2.8
NAA	0.5	100	4.8	2.3
	1.0	70	3.1	1.6
	2.0	40	2.1	1.4
CD			0.60	0.41

Table 3. Influence of hardening procedure on survival percentage of plantlets

Hardening steps	Survival % of plantlets	
	Vermiculite : Cocopeat (3:1)	Vermiculite : Vermicompost (3:1)
Two step procedure: In greenhouse for 10-12 days and subsequent transfer in shade house.	70	40
Three step procedure: In acclimatization hood kept in culture room for 10-12 days and subsequent transfer to Environment control greenhouse for 8-10 days, thereafter to shade house.	100	60

explants rinsing with water and soaking for 30 minutes in 0.2% Bavistin solution were also necessary steps of decontaminant procedures. The shoot tip after thorough rinsing with distilled water, were then subjected for surface sterilization with 3-4 minutes with 0.1% HgCl_2 solution under laminar flow hood. Explants were dissected and trimmed to the size (~1.0 cm) for inoculation onto different medium for micro-sucker proliferation.

In the present study, shoot tip explants were assessed for *in vitro* morphogenic response. The concentration and combination of plant growth regulator greatly influenced the responsive explants in terms of percentage of responsive culture and rate of micro-sucker proliferation. The highest percentage (100%) of explants were found morphogenic responsive when cultured on several medium composition as compared to medium containing higher concentration of BA alone or in combination of TDZ or NAA (as indicated in Table 1). Medium devoid of plant growth regulators could not show response for multiple sucker proliferation. After passing to three sub-cultures, the proliferation of micro-sucker was remarkably high (12 micro-shoots) in T_0 treatment (Table 1). The maximum mean length (2.3 cm) of micro-sucker was recorded in BA 1.0 mg + 0.5 mg TDZ + 0.1 mg NAA per litre combination.

Further, increase in concentration of cytokinin (BA and TDZ) alone or in combination of NAA reduced micro-sucker proliferation. The variation in proliferation of shoots and responsive culture was also reported by Saroha *et al.* (2005), Aggarwal and Barna (2004) and Chaudhari and Mukundan (2001). The results of these workers indicated that the combination of two cytokinins along with auxin either IAA, NAA or BA was superior as compared to addition of cytokinin alone. The study of Saroha *et al.* (2005) further confirmed that TDZ 1 mg/l was superior to BA in combination of Adenine Sulphate or NAA.

The culture with proliferated micro-suckers were subjected to hormone free medium for 2-3 weeks for elongation to get rootable size of micro-sucker. The cent per cent rooting was observed in micro-suckers cultured on 0.5 mg/l NAA. The longest root was achieved with 0.1 mg/l NAA. The response to all the concentrations of IAA was poor for root formation in terms of rooting percentage and number of roots and root length. *In vitro* rooting in aloeveira has been obtained by Liao *et al.* (2004), Ray and Sarkar (1991), Natali *et al.* (1990) with varied response of different auxins.

Hardening of plantlets

The success of any *in vitro* regeneration protocol largely depends on the survival and growth performance of propagated plantlets *ex vitro*. Moreover, under hot arid

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- agro-ecosystem, hardening and acclimatization of plantlets are very challenging component of micropropagation. (Singh *et al.*, 2005) In the present study a three step hardening facility (Table 4) was devised and maximum 100 per cent of plantlets were acclimatized successfully as compared to low survival rate (40%) under the two step procedure. This was achieved probably due to integration of acclimatization hood as one of the important hardening steps, which favorably supported the process and facilitated the desired temperature (25-30°C) and humidity (80-90%) suited to hardening during first stage of hardening which favourably supported the *in vitro* produced delicate roots in autotrophic media. In Aloe vera, Ahmed *et al.* (2007) recorded 82 per cent survival in tissue culture Aloe vera plantlets during acclimatization with mixture of garden soil, compost and sand in proportion 2:2:1. Similar results were also noted by Agarwal and Barna (2004). Preece and Sutter (1991) also emphasized that certain environmental conditions are necessary for acclimatization of plantlets using controlled environmental facilities.
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Response of pruning intensity and nutrients on vegetative growth parameters and yield of Aonla (*Emblica officinalis* Gaertn.) cv. Francis

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Abstract

Pruning is very effective to induce healthy current season growth. It also responds to slow growing trees by maintaining the size and shape without sacrificing yield. Further, proper nutrient management is also essential to maintain soil fertility and plant nutrient supply to an optimum level. The aonla plant responds the light as well as severe pruning. Thus, an investigation on response of pruning and nutrient management on vegetative growth and fruit yield has been conducted during the two consecutive years.

The maximum increase in vegetative growth characteristics, fruit yield and quality was found in 50% pruning intensity along with nutrient management by use of FYM (40 kg) and NPK (1000:500:1000 g/plant). Since, pruning remove carbon-starved, fruiting exhausted shoots and promotes new leaf growth to build up carbohydrate reserves for the next flowering that allows the sprouting of lateral buds hence, ultimately influenced the plant growth, spread, trunk girth and other vegetative characteristics of the plants. The significant interactive effect of treatments was attributed to the favourable nutritional status of the leaf and soil resulting into increased biomass production of the fruit. A significant increase in yield and yield parameters in aonla with nutrient application would have been due to vigorous vegetative growth and increased chlorophyll content, which together accelerated the photosynthesis that increased the supply of carbohydrates to plants.

Key words: aonla, pruning, antiscorbutic, senile, rejuvenation, photosynthates,

Introduction

Aonla (*Emblica officinalis* Gaertn.) is an important and highly remunerative arid fruit plant that grows under wide range of agro-climatic conditions without much care. The aonla fruit has been recognized throughout the world as health care drug, valued to restore vitality and remedy of a number of body ailments. The fair amount of polyphenols and tannins (gallic acid, elagic acid and glucose), found in fresh and dried aonla fruit, retard the oxidation of vitamin C and functions as antiscorbutic element.

In spite of being such a valuable crop, most of the old aonla orchards are declining in terms of productivity and quality of fruits due to sole reason of poor management practices. Hence, there is dire need to improve the health and productivity of such unproductive/senile orchards through application of rejuvenation technology, efficient management practices of nutrients, moisture management, organic farming and control over pests and diseases. With these objectives an investigation on response of pruning and nutrient management on vegetative growth and fruit

yield has been conducted during the two consecutive years, 2007 and 2008.

Materials and Methods

The field experiment was conducted at Main Experiment Station, Department of Horticulture, NDUAT, Faizabad, Uttar Pradesh. Thirty-six plants of sixteen years old aonla cv. Francis; uniform in size and growth, planted at a distance of 10×10 m were selected. The experiment was laid out in a Factorial Randomized Block Design with 3 replications having 12 treatment combinations including 3 levels of pruning (un-pruned, 25% and 50% removal of one year old shoot), 2 levels of organic (Bio-press mud 20 kg/tree and FYM 40 kg/tree) and inorganic nutrients (50% and 100% RDF).

The full dose of FYM, bio-press mud, phosphorus, potassium and half dose of nitrogen were applied as a basal dose in the month of February, while another 1/2 dose nitrogen was applied after fruit bud bursting in the month of September. Pruning was done in the second week of February. Observations were recorded on the plant height, spread, trunk girth, number of determinate and indeterminate shoots per pruned and un-

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pruned shoots, days taken to sprouting, per cent fruit set, days taken to fruit maturity and yield.

Results and discussion

Effect of pruning intensity on vegetative growth parameters

The data presented in the Table-1(a) and (b) showed that plant height, determinate and indeterminate shoots and days taken to sprouting was significantly influenced by pruning intensity in both the years of experimentation while plant spread was influenced only in 2008. The maximum increase in plant height was recorded in P_2 (50% pruning of previous season growth) 14.31 and 14.00 per cent and minimum in P_0 (control) 11.81 and 10.58 per cent during 2007 and 2008 respectively. The maximum plant spread was found to be 12.79 per cent in P_2 and minimum 9.98 per cent in P_0 during 2008 but did not leave much effect in the year 2007. Application of various pruning intensity did not show significant influence on girth of trunk during both the years of experimentation.

Further, significantly maximum number of the determinate shoots (35.75, 34.67) and indeterminate shoots (6.06, 6.02) were observed under P_0 as compared to minimum determinate and indeterminate shoots under P_2 (27.50, 28.58) and (3.70, 3.92) during both years respectively. The maximum days taken to sprouting was obtained under P_2 (11.67 and 13.25 days) as compared to P_0 (7.75 and 5.75 days) in the years 2007 and 2008 respectively.

Effect of organic manures and nutrient management on vegetative growth parameters

A perusal of data presented in Table-1(a) reveal that application of organic manures and nutrients did not show significant influence on plant height, spread, girth of trunk and indeterminate shoots during both the years of experimentation. Nevertheless, application of nutrients through 100 % RDF (1000:500:1000 g/tree NPK) and 50 % RDF (500:250:500 g/tree NPK) left significant effect on increase of trunk girth (17.17 per cent) and minimum days taken to sprouting (10.17 days) during 2008. Again, application of recommended dose of nutrients showed significant effect on number of the determinate shoots were noticed under N_2 (34.67 in both years) and minimum under N_1 (28.78, 29.11) during 2007 and 2008 respectively.

Effect of interaction treatments on vegetative growth parameters

The data related to effect of interaction treatments on plant height, spread, trunk girth, determinate shoot, indeterminate shoot and days taken to sprouting are presented in Table-1(a) and (b). There was significant maximum increase in plant height (16.00 and 16.67 per cent) under $P_2M_2N_1$ and $P_2M_2N_2$ and minimum (10.83 and 10.16 per cent) under $P_0M_1N_1$ and $P_0M_1N_2$ was found during 2007 and 2008, respectively. Interaction effect on plant spread is

significant in 2008 with maximum and minimum values (13.92 and 9.82 per cent) under $P_2M_1N_2$ and $P_0M_2N_2$, respectively. Trunk girth was insignificantly influenced during both the years.

The significantly maximum number of the determinate shoots (40.00 and 38.33) under $P_0M_2N_2$ and minimum (24.00 and 25.33) under $P_2M_2N_1$ were noticed during 2007 and 2008, respectively. Further, maximum number of the indeterminate shoots (6.75) under $P_0M_2N_2$ in 2007 and (6.3) under $P_0M_1N_1$ and $P_0M_2N_1$ during 2008 and minimum (2.98 and 1.30) under $P_2M_2N_1$ and $P_2M_1N_1$ was noticed during 2007 and 2008 respectively. The minimum days (6.7 and 5.0 days) were taken for sprouting with treatment $P_0M_1N_1$ and $P_0M_2N_2$, respectively whereas, maximum (13.7 and 15.0 days) under $P_2M_2N_1$ during both years 2007 and 2008.

Since, pruning remove carbon-starved, fruiting exhausted shoots and promotes new leaf growth to build up carbohydrate reserves for the next flowering that allows the sprouting of lateral buds hence, ultimately influenced the plant growth, spread, trunk girth and other vegetative characters of the plants. The findings are in accordance with Dhaliwal *et al.* (2000) in guava and Singh (2005) in aonla.

The increase in plant height, spread, number of determinate and indeterminate shoots would have been caused due to the acceleration of the movement of assimilates and increase in translocation of photosynthates as a result of NPK application (Hartt, 1969; Teatota *et al.*, 1972 in guava and Glonti, 1972 in orange) whereas, the result of combined use of fertilizers and organic manures are in close agreement with the findings of Santhy *et al.* (2001) who reported that integrated nutrient supply increased organic carbon, available nitrogen, sulphur, manganese and iron improving soil fertility.

Effect of pruning intensity on yield parameters

It is evident from the data presented in Table-2 that irrespective of any treatment, the initiation of the flowering in aonla started as early as on March 23 and latest by March 30 during 2007 and from March 22 to March 31 in 2008. It is crystal clear from the data that the response of pruning on fruit set, duration of fruit maturity and fruit yield had been significant in both years. Maximum fruit set (71.74 and 70.54 per cent) and fruit yield (125 and 130 kg/tree) was noticed in P_2 whereas minimum (58.37 and 57.72 per cent) and (98 and 100 kg/tree) in P_0 . Further, the minimum days taken to fruit maturity (218 days in both years) was also found to be in P_2 as compared to P_0 (228 and 229 days).

Effect of organic manures and nutrient management on yield parameters

The result obtained under present investigation presented in Table-2 reveals that application of organic and inorganic nutrient management significantly improve

Table-1(a). Effect of the pruning intensity, organic manure and nutrient management on plant growth characteristics (per cent increase over initial plant growth)

Treatments	Plant height (%)		Plant spread (%)		Trunk girth (%)	
	2007	2008	2007	2008	2007	2008
Pruning						
P ₀	11.81	10.58	09.33	09.98	16.92	14.25
P ₁	13.51	12.42	09.30	10.57	18.00	17.08
P ₂	14.31	14.00	09.83	12.79	18.33	15.83
SEm ±	0.286	0.381	0.381	0.423	1.105	1.169
CD at 5%	0.837	1.111	NS	1.239	NS	NS
Organic Manures						
M ₁	12.89	11.92	09.31	10.83	18.45	15.78
M ₂	13.53	12.77	09.66	11.40	17.06	15.67
SEm ±	0.234	0.310	0.310	0.346	0.902	0.954
CD at 5%	NS	NS	NS	NS	NS	NS
Nutrients						
N ₁	13.23	12.11	09.62	10.91	16.84	14.28
N ₂	13.19	12.58	09.35	11.32	18.67	17.17
SEm ±	0.234	0.310	0.310	0.346	0.902	0.954
CD at 5%	NS	NS	NS	NS	NS	2.792
Interaction						
P ₀ M ₁ N ₁	10.83	10.67	09.17	10.10	16.67	12.67
P ₀ M ₁ N ₂	12.50	10.16	08.88	10.06	19.00	15.67
P ₀ M ₂ N ₁	11.90	10.93	09.47	09.93	14.67	13.00
P ₀ M ₂ N ₂	12.00	10.67	09.80	09.82	17.33	15.67
P ₁ M ₁ N ₁	13.47	13.00	09.97	09.93	17.67	16.00
P ₁ M ₁ N ₂	14.00	12.33	08.32	10.97	20.00	18.67
P ₁ M ₂ N ₁	13.63	11.67	09.77	10.44	18.23	15.00
P ₁ M ₂ N ₂	12.95	12.67	09.13	10.92	16.00	18.67
P ₂ M ₁ N ₁	13.57	12.33	09.58	11.71	18.00	15.33
P ₂ M ₁ N ₂	13.00	13.00	09.97	12.19	19.33	16.33
P ₂ M ₂ N ₁	16.00	14.00	09.78	13.32	15.67	13.66
P ₂ M ₂ N ₂	14.67	16.67	10.00	13.92	20.33	18.00
SEm ±	0.572	0.759	0.761	0.847	2.210	2.337
CD at 5%	1.674	2.222	NS	2.481	NS	NS

the flowering attributes, fruit set and fruit maturity period during both the years except fruit yield only during 2008.

The maximum fruit set (66.80 and 65.95 per cent), fruit yield (115 and 119 kg/tree) and minimum days taken to fruit maturity (222 days in both years) were found under organic manure (M₂) whereas minimum (60.84 and 60.07 per cent), (108 and 110 kg/tree) and maximum (224 days, 226 days) under M₁ during both years 2007 and 2008 respectively. Similarly, the maximum fruit set (64.89 and 64.50 per cent), fruit yield (115 and 118 kg/tree) and minimum days taken to fruit maturity in M₂ (221 and 222 days) was

noticed under nutrients N₂ and minimum (62.78 and 61.52 per cent), (109 and 111 kg/tree) and maximum (225 days and 226 days) due to the treatment N₁.

Effect of interaction treatments on yield parameters:

The significant effect was observed due to interaction of different levels of pruning intensity, organic manure and nutrients on fruit set, days taken to maturity and fruit yield.

The earliest flower initiation was started from March 23 recorded in P₀M₂N₂ during 2007 and from March

Table-1(b). Effect of pruning intensity, organic manure and nutrient management on plant growth characteristics

Treatments	No. of determinate shoots		No. of indeterminate shoot (Days)		Days taken to sprouting	
	2007	2008	2007	2008	2007	2008
Pruning						
P0	35.75	34.67	06.06	06.02	07.75	05.75
P1	31.92	32.42	04.60	04.88	08.42	08.75
P2	27.50	28.58	03.70	03.92	11.67	13.25
SEm ±	0.953	1.086	0.293	0.297	0.736	0.536
CD at 5%	2.788	3.177	0.857	0.868	2.154	1.568
Organic Manure						
M1	31.94	31.89	04.62	04.51	08.72	09.17
M2	31.50	31.89	04.96	05.37	09.83	09.33
SEm ±	0.778	0.887	0.239	0.242	0.601	0.438
CD at 5%	NS	NS	NS	NS	NS	NS
Nutrients						
N1	28.78	29.11	04.76	04.76	09.39	10.17
N2	34.67	34.67	04.82	05.13	09.17	08.33
SEm ±	0.778	0.887	0.239	0.242	0.601	0.438
CD at 5%	2.276	2.594	NS	NS	NS	1.658
Interactions						
P0M1N1	35.00	34.33	06.00	06.30	06.70	05.00
P0M1N2	38.00	36.00	05.00	06.00	07.30	06.00
P0M2N1	30.00	30.00	06.50	06.30	08.00	07.00
P0M2N2	40.00	38.33	06.75	05.40	09.00	05.00
P1M1N1	28.67	29.00	04.55	04.70	09.00	10.00
P1M1N2	34.00	34.67	04.60	05.00	08.30	08.00
P1M2N1	28.00	28.33	04.50	04.67	09.00	10.00
P1M2N2	37.00	37.67	04.75	05.16	07.30	07.00
P2M1N1	27.00	27.67	04.00	01.30	10.00	14.00
P2M1N2	29.00	29.67	03.57	03.76	11.00	12.00
P2M2N1	24.00	25.33	02.98	05.20	13.70	15.00
P2M2N2	30.00	31.67	04.30	05.40	12.00	12.00
SEm ±	1.906	2.171	0.586	0.593	1.473	1.072
CD at 5%	5.57	66.3531.713	1.736	4.308		

22 in 2008 in P₀M₁N₁ treatment. The late flower initiation was seen in P₂M₂N₂ (March 30) during 2007 and P₂M₂N₂ in 2008 (March 31). It is also evident that with increase in pruning intensity time of flower initiation was delayed.

Maximum fruit set (74.92 and 74.61 per cent) and fruit yield (139 and 145 kg/tree) was noticed in P₂M₂N₂ and minimum fruit set (53.38 and 53.18 per cent) and (88 and 89 kg/tree) in P₀M₁N₁ during the years 2007 and 2008, respectively. Further, minimum days of maturity period were noticed under P₂M₂N₂ (215 and 216 days) and maximum days under P₀M₁N₁ (232 and 237 days) during 2007 and 2008, respectively.

Chandra and Govind (1995) found maximum fruit yield (9.18 kg/tree) with 75 per cent pruning in the month of February whereas, good quality was obtained with 25 per cent pruning in guava. Kundu *et al.* (1995) also found that yields were reduced significantly as the pruning severity was increased. Mishra and Pathak (1998) reported that 50 per cent pruning in May produced highest yield (25.8 kg/tree) than un-pruned (7.6 kg/tree) in winter crop of guava cv. Sardar.

However, other factors like cultural practices and nutrition of the plants also influenced flowering characters to an appreciable extent, (Tripathi and Maity 2007). The

Table-2. Effect of pruning intensity, organic manure and nutrient management on flowering and fruiting characteristics

Treatments	Time of flower initiation (Date)		Fruit set (%)		Days taken to fruit maturity (Days)		Fruit yield (kg/tree)	
	2007	2008	2007	2008	2007	2008	2007	2008
Pruning								
P ₀	Mar.25	Mar.25	58.37	57.72	228	229	98	100
P ₁	Mar.27	Mar.27	61.34	60.78	223	224	111	114
P ₂	Mar.29	Mar.30	71.74	70.54	218	218	125	130
SEm ±	-	-	0.235	0.850	0.783	0.399	2.326	1.384
CD at 5%	-	-	0.687	2.487	2.291	1.166	6.804	4.050
Organic manure								
M ₁	Mar.27	Mar.26	60.84	60.07	224	226	108	110
M ₂	Mar.27	Mar.28	66.80	65.95	222	222	115	119
SEm ±	-	-	0.192	0.694	0.639	0.325	1.91	1.130
CD at 5%	-	-	0.561	2.031	1.870	0.952	NS	3.306
Nutrients								
N ₁	Mar.27	Mar.27	62.78	61.52	225	226	109	111
N ₂	Mar.26	Mar.27	64.89	64.50	221	222	115	118
SEm ±	-	-	0.192	0.694	0.639	0.325	1.91	1.130
CD at 5%	-	-	0.561	2.031	1.870	0.952	NS	3.306
Interactions								
P ₀ M ₁ N ₁	Mar.24	Mar.22	53.38	53.18	232	237	88	89
P ₀ M ₁ N ₂	Mar.26	Mar.25	54.67	54.39	225	225	98	98
P ₀ M ₂ N ₁	Mar.26	Mar.27	62.00	59.67	227	227	102	105
P ₀ M ₂ N ₂	Mar.23	Mar.24	63.41	63.63	228	228	105	108
P ₁ M ₁ N ₁	Mar.26	Mar.25	57.45	55.81	227	227	110	110
P ₁ M ₁ N ₂	Mar.27	Mar.28	59.45	61.48	223	224	112	115
P ₁ M ₂ N ₁	Mar.28	Mar.27	61.43	60.46	222	223	111	113
P ₁ M ₂ N ₂	Mar.25	Mar.26	67.03	65.34	221	222	112	116
P ₂ M ₁ N ₁	Mar.29	Mar.28	70.39	67.98	223	224	117	121
P ₂ M ₁ N ₂	Mar.28	Mar.29	69.67	67.56	216	216	122	127
P ₂ M ₂ N ₁	Mar.30	Mar.30	72.00	72.00	217	218	123	125
P ₂ M ₂ N ₂	Mar.29	Mar.31	74.92	74.61	215	216	139	145
SEm ±	-	-	0.470	1.700	1.566	0.797	4.651	2.768
CD at 5%	-	-	1.374	4.974	4.581	2.332	13.608	8.099

application of organic manures might have increased the production of organic acids which play a leading role in availability of P (Mukherjee *et al.*, 1991) that would have induced early flowering than its counter part. Organic manures attributes favourable effect on microbial and root proliferation in soil, which caused solubilizing effect on native N, P, K and other nutrients (Nambiar, 1994).

Combined application of organic manures and fertilizers favour vigorous growth and synthesized more cytokinin in plants which might have helped in translocation of this synthesized cytokinin as well as more quantity of available phosphorus through the xylem vessels and the accumulation of cytokinin and phosphorus in these axillary buds would have favoured the plant to enter into reproductive phase (Dange *et al.*, 2002).

A significant increase in yield and yield parameters in aonla with nutrient application would have been due to vigorous vegetative growth and increased chlorophyll content, which together accelerated the photosynthesis that increased the supply of carbohydrates to plants. The beneficial role of supplemented organic manures in improving soil physical, chemical and biological activities is well known, that helped in better nutrient absorption by plants, resulting higher yield (Prabu *et al.*, 2002).

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Studies on micronutrient cations in arid irrigated aonla orchard soil profiles in Sikar district of Rajasthan

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Abstract

The present study on distribution of micronutrient cations in arid irrigated aonla orchard soil profiles in Sikar district of Rajasthan was conducted during March 2007-2008. The study included analysis of soil profiles with respect to micronutrients. The available iron ranged between 2.28 to 6.51 mg kg⁻¹, available zinc between 0.203 to 0.990 mg kg⁻¹, available copper between 0.110 to 0.493 mg kg⁻¹ and available manganese between 1.36 to 5.19 mg kg⁻¹. All the micronutrients were found to be low to medium in range in arid irrigated aonla orchard soil profiles in Sikar district of Rajasthan.

Key words: Aonla, Orchards, Micronutrients

Introduction

Aonla is an important fruit crop which has high nutritive and medicinal value. It is one of the richest source of vitamin 'C' ranging from 500 to 1500 mg/100 g pulp. Fruits are also rich in pectin and minerals like iron, calcium, phosphorus. The aonla has been recommended by Ayurveda for balanced diet and sound health and is important ingredient of triphala and chavanprash. Due to multipurpose uses, the demand of aonla fruits is increasing day by day. Because of wide adaptive nature, this fruit crop is mainly grown on dry land and waste land soils where major nutrients are deficient and limited irrigation facilities are available. Its cultivation has become quite popular in arid irrigated regions of Rajasthan, owing to its high nutritive and medicinal value and having higher productivity even in the wasteland. It is also a value added horticultural crop which has bright future prospects for export, particularly to European countries. In Indian fruit industry, poor nutrition is the major cause of low orchard efficiency resulting poor productivity and poor fruit quality. Sufficient information on nutrient management in fruit crops has been generated, but response and requirement of nutrients of perennial fruit crops vary markedly in a particular area depending on soil and climatic conditions and also depend on growth, bearing habit, age, root stock

and management practices. Balanced nutrition of fruit crops is paramount importance particularly in arid areas having largely sandy soils of the orchards for their fertility status is done. For knowing the exact status of mineral nutrition in the fruit trees, survey of the orchards for their fertility status and plant nutrients is done. Studies on nutritional survey of arid fruits in Rajasthan are meagre inspite of their great importance and relevance. With this aim, the present study was undertaken to evaluate the micronutrient fertility status of different orchards in Sikar district of Rajasthan.

Materials and Methods

The present investigation were carried out in Sikar district at the orchards of 8 aonla fruit growers spread over the whole district during the year 2007-2008. Orchards were selected in such a way that these can represent general conditions of area under study. The details of locations and name of the fruit growers are given in table.

S.No.	Name of fruit grower	Village	location
1.	Sh. Balbir Singh	Rashidpura	Rashidpura
2.	Shri Dana Ram	Rehnawa	Rehnawa
3.	Shri Raju Sen	Laxmangarh	Laxmangarh
4.	Shri Purn Mal Shartma	Sargoth	Sargoth
5.	Shri Roopchand Pipaliwal	Pachar	Pachar I
6.	Shri Kana Ram	Pachar	Pachar II
7.	Shri Panna Ram	Pachar	Pachar VI
8.	Govt. Nursery	Palsana	Palsana I

Soil samples were collected from eight fruit orchards, on the basis of variability and orchard performance at different locations of Sikar district. Thereafter, from each selected orchards, three soil profiles, based on soil fertility variation and plant performance were taken up. One hundred ninety two representative composite soil samples at different soil depths viz., 0-15, 15-30, 30-45, 45-60, 60-75, 75-90, 90-105 and 105-120 cm. were collected. After processing, the soil samples were analyzed for micronutrients. The available Fe, Cu, Zn, and Mn were extracted with DTPA solution as per procedure of Lindsay and Norvell (1978) and were determined by atomic absorption spectrophotometry.

Results and discussion

The distribution of DTPA extractable micronutrients as depth wise in 8 aonla fruit orchards in Sikar district of Rajasthan was studied.

DTPA extractable Iron: The extractable iron in soils ranged from 2.28 to 6.51 mg kg⁻¹ which showed a regular decreasing trend with increasing depths. It is evident that 50 per cent soil samples drawn from surface soil depth of aonla orchards were deficient and remaining 50 per cent were sufficient in DTPA extractable iron in the present study. The deficient to sufficient status of iron found in the soil might be due to calcareousness, low organic carbon content, light textured coarse sandy soils. Lal and Biswas (1973) observed that available iron content ranged from 0.3 to 5.6 ppm in Rajasthan soils and it was low i.e. 2 ppm in the desert soils, whereas, similar observations were reported by Kumawat (2005) and Rohitash (2007). These finding are in conformity to the results of present investigation.

DTPA extractable zinc: The DTPA extractable zinc content in the soils ranged from 0.203 to 0.990 with the mean value of 0.587 of all the soil depths. Taking 0.60 mg kg⁻¹ as critical limit given by Tandon, 1992, 50 per cent soil samples drawn from surface soil depth of aonla orchards were deficient and remaining 50 per cent were sufficient in with respect to available zinc content and also showed a regular decreasing trend with increase in depth. The deficiency of zinc in soils might be due to the presence of quartz, feldspar or the exchange complex being such as to have sites saturated with Ca/ Mg under alkaline soil reaction. Calcareous nature and low organic matter are some of the other properties where low levels of zinc are anticipated. These results are in accordance with the findings of Bhatnagar and Chandra (2003), Kumawat (2005) and Rohitash (2007).

DTPA extractable copper: It is evident that the DTPA extractable copper content of soil depths ranged from 0.110 to 0.493 mg kg⁻¹ with their mean value 0.238 mg kg⁻¹. The copper content showed a regular decreasing trend with increase in depth. The 50 per cent soil samples drawn from surface soil depth of aonla orchards were deficient and remaining 50 per cent were sufficient in with respect to

available copper content. The deficient to sufficient available copper status of orchard soil might be due to high pH, calcareousness, lower organic carbon and light textured coarse sandy soils. The present results are in accordance with those reported by Baser and Lodha (1971) who reported that available copper status in sandy soils of Rajasthan varied from 0.05 to 2.38 ppm. Similar types of results have also been reported by Bhatnagar and Chandra (2003) who reported available copper status in sandy soils of Rajasthan varied from 0.08 to 0.51 ppm.

DTPA extractable manganese: The data presented on DTPA extractable manganese content of orchard soils showed a regular decreasing trend with increase in depths. The DTPA extractable manganese content in the soils ranged from 1.36 to 5.19 mg kg⁻¹ with their mean value of 3.00 of all the soil depths. Twenty five per cent soil samples of aonla were found deficient and remaining 75 per cent were sufficient in respect to DTPA extractable manganese. Results of DTPA extractable manganese in all the orchards clearly indicate that deficiency of manganese in the aonla orchards is now coming. The deficiency of DTPA extractable manganese might be due to the presence of high CaCO₃ content and low organic carbon content. These results are in accordance with those reported by Bhatnagar and Chandra (2003) and Kumawat (2005).

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Table 1. DTPA Extractable Iron (mg kg^{-1}) in Aonla Orchards at Different Soil Depths in Sikar District

Location of orchards	Soil depths (cm)								Mean
	0-15	15-30	30-45	45-60	60-75	75-90	90-105	105-120	
Rashidpura	2.50 $\pm$ 0.34	2.47 $\pm$ 0.10	2.46 $\pm$ 0.09	2.44 $\pm$ 0.08	2.41 $\pm$ 0.08	2.38 $\pm$ 0.08	2.32 $\pm$ 0.08	2.28 $\pm$ 0.51	2.41 $\pm$ 0.24
Rehnawa	4.24 $\pm$ 0.05	4.22 $\pm$ 0.05	4.20 $\pm$ 0.05	4.16 $\pm$ 0.07	4.12 $\pm$ 0.06	4.08 $\pm$ 0.06	4.04 $\pm$ 0.06	3.99 $\pm$ 0.37	4.13 $\pm$ 0.17
Laxmangarh	3.34 $\pm$ 0.04	3.32 $\pm$ 0.86	3.29 $\pm$ 0.05	3.22 $\pm$ 0.07	3.20 $\pm$ 0.07	3.16 $\pm$ 0.06	3.10 $\pm$ 0.07	3.05 $\pm$ 0.35	3.21 $\pm$ 0.34
Sargoth	3.83 $\pm$ 0.04	3.81 $\pm$ 0.05	3.78 $\pm$ 0.77	3.71 $\pm$ 0.00	3.72 $\pm$ 0.07	3.67 $\pm$ 0.07	3.63 $\pm$ 0.06	3.60 $\pm$ 0.40	3.72 $\pm$ 0.32
Pachar-I	4.65 $\pm$ 0.05	4.62 $\pm$ 0.04	4.58 $\pm$ 0.05	4.54 $\pm$ 0.04	4.49 $\pm$ 0.06	4.47 $\pm$ 0.06	4.41 $\pm$ 0.84	4.71 $\pm$ 1.70	4.56 $\pm$ 0.68
Pachar-II	5.37 $\pm$ 0.04	5.35 $\pm$ 0.04	5.32 $\pm$ 0.04	5.31 $\pm$ 0.00	5.23 $\pm$ 0.84	5.18 $\pm$ 0.05	5.14 $\pm$ 0.05	5.07 $\pm$ 0.42	5.25 $\pm$ 0.35
Pachar-VI	6.51 $\pm$ 0.10	6.48 $\pm$ 0.10	6.48 $\pm$ 0.08	6.44 $\pm$ 0.07	6.38 $\pm$ 0.07	6.35 $\pm$ 0.06	6.30 $\pm$ 0.03	6.25 $\pm$ 0.38	6.40 $\pm$ 0.17
Palsana-I	6.08 $\pm$ 0.06	6.06 $\pm$ 0.06	5.99 $\pm$ 0.08	5.97 $\pm$ 0.08	5.89 $\pm$ 0.09	5.86 $\pm$ 0.09	5.81 $\pm$ 0.09	5.77 $\pm$ 0.32	5.93 $\pm$ 0.17
Mean	4.56	4.54	4.51	4.47	4.43	4.39	4.34	4.34	

$\pm$ indicates standard deviation, Deficient - 50%, Sufficient - 50%

Table 2. DTPA Extractable Zinc (mg kg^{-1}) in Aonla Orchards at Different Soil Depths in Sikar District

Location of orchards	Soil depths (cm)								Mean
	0-15	15-30	30-45	45-60	60-75	75-90	90-105	105-120	
Rashidpura	0.357 $\pm$ 0.021	0.340 $\pm$ 0.024	0.313 $\pm$ 0.021	0.293 $\pm$ 0.025	0.270 $\pm$ 0.028	0.237 $\pm$ 0.033	0.233 $\pm$ 0.017	0.203 $\pm$ 0.025	0.281 $\pm$ 0.057
Rehnawa	0.560 $\pm$ 0.051	0.537 $\pm$ 0.056	0.520 $\pm$ 0.057	0.497 $\pm$ 0.056	0.470 $\pm$ 0.054	0.440 $\pm$ 0.059	0.413 $\pm$ 0.057	0.387 $\pm$ 0.058	0.478 $\pm$ 0.080
Laxmangarh	0.413 $\pm$ 0.017	0.393 $\pm$ 0.021	0.370 $\pm$ 0.029	0.347 $\pm$ 0.033	0.313 $\pm$ 0.025	0.293 $\pm$ 0.025	0.283 $\pm$ 0.025	0.260 $\pm$ 0.037	0.334 $\pm$ 0.058
Sargoth	0.653 $\pm$ 0.045	0.630 $\pm$ 0.043	0.603 $\pm$ 0.039	0.577 $\pm$ 0.042	0.550 $\pm$ 0.036	0.530 $\pm$ 0.036	0.513 $\pm$ 0.039	0.493 $\pm$ 0.039	0.569 $\pm$ 0.067
Pachar-I	0.850 $\pm$ 0.043	0.827 $\pm$ 0.047	0.810 $\pm$ 0.050	0.797 $\pm$ 0.048	0.773 $\pm$ 0.045	0.750 $\pm$ 0.043	0.730 $\pm$ 0.043	0.707 $\pm$ 0.045	0.780 $\pm$ 0.065
Pachar-II	0.953 $\pm$ 0.033	0.933 $\pm$ 0.033	0.913 $\pm$ 0.033	0.897 $\pm$ 0.029	0.873 $\pm$ 0.033	0.857 $\pm$ 0.042	0.840 $\pm$ 0.037	0.817 $\pm$ 0.033	0.885 $\pm$ 0.056
Pachar-VI	0.530 $\pm$ 0.057	0.503 $\pm$ 0.042	0.483 $\pm$ 0.042	0.460 $\pm$ 0.185	0.440 $\pm$ 0.037	0.420 $\pm$ 0.045	0.397 $\pm$ 0.046	0.517 $\pm$ 0.061	0.469 $\pm$ 0.091
Palsana-I	0.990 $\pm$ 0.016	0.973 $\pm$ 0.012	0.953 $\pm$ 0.012	0.927 $\pm$ 0.017	0.903 $\pm$ 0.021	0.880 $\pm$ 0.024	0.843 $\pm$ 0.026	0.770 $\pm$ 0.086	0.905 $\pm$ 0.077
Mean	0.663	0.642	0.621	0.599	0.574	0.551	0.532	0.519	

$\pm$ indicates standard deviation, Deficient - 50%, Sufficient - 50%

Table 3. DTPA Extractable Copper (mg kg^{-1}) in Aonla Orchards at Different Soil Depths in Sikar District

Location of orchards	Soil depths (cm)								Mean
	0-15	15-30	30-45	45-60	60-75	75-90	90-105	105-120	
Rashidpura	0.177 $\pm$ 0.039	0.167 $\pm$ 0.031	0.157 $\pm$ 0.031	0.143 $\pm$ 0.026	0.153 $\pm$ 0.026	0.143 $\pm$ 0.026	0.133 $\pm$ 0.012	0.120 $\pm$ 0.008	0.149 $\pm$ 0.032
Rehnawa	0.190 $\pm$ 0.041	0.173 $\pm$ 0.029	0.163 $\pm$ 0.021	0.153 $\pm$ 0.021	0.143 $\pm$ 0.012	0.137 $\pm$ 0.017	0.137 $\pm$ 0.009	0.127 $\pm$ 0.009	0.153 $\pm$ 0.030
Laxmangarh	0.193 $\pm$ 0.029	0.183 $\pm$ 0.029	0.167 $\pm$ 0.012	0.153 $\pm$ 0.009	0.147 $\pm$ 0.005	0.137 $\pm$ 0.005	0.130 $\pm$ 0.000	0.120 $\pm$ 0.000	0.154 $\pm$ 0.029
Sargoth	0.177 $\pm$ 0.012	0.163 $\pm$ 0.017	0.150 $\pm$ 0.008	0.140 $\pm$ 0.008	0.130 $\pm$ 0.000	0.123 $\pm$ 0.005	0.120 $\pm$ 0.000	0.110 $\pm$ 0.000	0.139 $\pm$ 0.023
Pachar - I	0.333 $\pm$ 0.012	0.313 $\pm$ 0.012	0.297 $\pm$ 0.009	0.280 $\pm$ 0.008	0.257 $\pm$ 0.009	0.237 $\pm$ 0.009	0.217 $\pm$ 0.009	0.190 $\pm$ 0.014	0.265 $\pm$ 0.047
Pachar - II	0.367 $\pm$ 0.040	0.350 $\pm$ 0.043	0.333 $\pm$ 0.045	0.313 $\pm$ 0.045	0.293 $\pm$ 0.045	0.273 $\pm$ 0.045	0.250 $\pm$ 0.043	0.220 $\pm$ 0.043	0.300 $\pm$ 0.064
Pachar - VI	0.493 $\pm$ 0.012	0.467 $\pm$ 0.012	0.443 $\pm$ 0.009	0.420 $\pm$ 0.008	0.407 $\pm$ 0.012	0.377 $\pm$ 0.017	0.357 $\pm$ 0.017	0.330 $\pm$ 0.016	0.412 $\pm$ 0.054
Palsana - I	0.430 $\pm$ 0.016	0.410 $\pm$ 0.016	0.383 $\pm$ 0.017	0.363 $\pm$ 0.009	0.330 $\pm$ 0.008	0.300 $\pm$ 0.008	0.270 $\pm$ 0.008	0.243 $\pm$ 0.012	0.341 $\pm$ 0.064
Mean	0.295	0.278	0.262	0.246	0.233	0.216	0.202	0.183	

$\pm$ indicates standard deviation, Deficient - 50%, Sufficient - 50%

Table 4. DTPA Extractable Manganese (mg kg^{-1}) in Aonla Orchards at Different Soil Depths in Sikar District

Location of orchards	Soil depths (cm)								Mean
	0-15	15-30	30-45	45-60	60-75	75-90	90-105	105-120	
Rashidpura	1.84 $\pm$ 0.05	1.69 $\pm$ 0.09	1.62 $\pm$ 0.08	1.57 $\pm$ 0.09	1.51 $\pm$ 0.09	1.46 $\pm$ 0.10	1.42 $\pm$ 0.11	1.37 $\pm$ 0.12	1.56 $\pm$ 0.17
Rehnawa	1.94 $\pm$ 0.02	1.71 $\pm$ 0.18	1.64 $\pm$ 0.20	1.57 $\pm$ 0.21	1.51 $\pm$ 0.21	1.48 $\pm$ 0.61	1.42 $\pm$ 0.22	1.36 $\pm$ 0.23	1.58 $\pm$ 0.33
Laxmangarh	2.25 $\pm$ 0.81	2.19 $\pm$ 0.00	2.11 $\pm$ 0.00	2.04 $\pm$ 0.01	1.99 $\pm$ 0.01	1.93 $\pm$ 0.02	1.89 $\pm$ 0.03	1.81 $\pm$ 0.02	2.03 $\pm$ 0.32
Sargoth	2.84 $\pm$ 0.02	2.76 $\pm$ 0.02	2.70 $\pm$ 0.02	2.64 $\pm$ 0.02	2.59 $\pm$ 0.02	2.52 $\pm$ 0.01	2.49 $\pm$ 0.02	2.44 $\pm$ 0.03	2.62 $\pm$ 0.13
Pachar - I	3.46 $\pm$ 0.02	3.40 $\pm$ 0.01	3.33 $\pm$ 0.03	3.27 $\pm$ 0.03	3.21 $\pm$ 0.02	3.15 $\pm$ 0.02	3.10 $\pm$ 0.01	3.01 $\pm$ 0.04	3.24 $\pm$ 0.15
Pachar - II	3.80 $\pm$ 0.03	3.70 $\pm$ 0.05	3.63 $\pm$ 0.82	3.56 $\pm$ 0.05	3.52 $\pm$ 0.06	3.45 $\pm$ 0.04	3.39 $\pm$ 0.03	3.32 $\pm$ 0.01	3.55 $\pm$ 0.33
Pachar - VI	5.19 $\pm$ 0.02	5.09 $\pm$ 0.07	5.01 $\pm$ 0.08	4.95 $\pm$ 0.07	4.90 $\pm$ 0.08	4.84 $\pm$ 0.09	4.78 $\pm$ 0.10	4.73 $\pm$ 0.11	4.94 $\pm$ 0.17
Palsana - I	4.76 $\pm$ 0.80	4.66 $\pm$ 0.82	4.60 $\pm$ 0.01	4.55 $\pm$ 0.03	4.50 $\pm$ 0.02	4.43 $\pm$ 0.03	4.26 $\pm$ 0.14	4.31 $\pm$ 0.00	4.51 $\pm$ 0.44
Mean	3.26	3.15	3.08	3.02	2.97	2.91	2.84	2.79	

$\pm$ indicates standard deviation, Deficient - 25%, Sufficient - 75%

Effect of CaCl_2 and GA_3 on shelf life of aonla (*Emblica officinalis* Gaertn) fruits

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Abstract

The Chakaiya variety of aonla was pre harvest sprayed with CaCl_2 , GA_3 and their combinations. Full mature fruits were harvested and stored at ambient temperature ($7.48-21.34^\circ\text{C}$). The findings suggest that fruits can be stored at ambient temperature for 5 days without any treatment whereas shelf life can be prolonged upto 20 days with 15.62 per cent decay loss by spraying 2% CaCl_2 + 100 ppm GA_3 25 days before harvesting of the fruits. The lowest PLW, higher T.S.S., maximum retention of ascorbic acid, higher reducing sugar, non-reducing sugar, total sugars and phenols were also estimated in 2% CaCl_2 + 100 ppm GA_3 treated fruits.

Key Words: *Emblica*, aonla, pre harvest spraying, nutrients, shelf life.

Introduction

Aonla or Indian gooseberry (*Emblica officinalis* Gaertn) is one of the most important indigenous fruit of India. It is very hardy fruit which can be successfully grown in various agro-climatic and soil conditions on which usual fruit crops do not perform well. In addition to India naturally growing aonla trees have also been reported from Ceylon, Cuba, Hawaii, Florida, Iran, Iraq, Java, West-Indies, Trinidad, Pakistan, Malaya and China. It is one of the richest source of vitamin 'C' (500-700 mg/100 g pulp), minerals and phenolic compounds and well known for its nutraceutical and pharmacological properties (Chatterjee and Sil, 2007; Calixto *et al.*, 1998) and is a wonderful antioxidant (Kumar *et al.*, 2006). The famous Indian Ayurvedic medicines chyavanprash and Trifla are prepared from aonla fruits. It is processed into quality product such as preserve, pickle, beverage, powder, Jam, etc. Due to its nutritive, medicinal and antioxidant properties aonla fruits demand is increasing in processing, pharmaceutical and cosmetic industries subsequently the area under aonla cultivation is getting expansion in Indian states like Uttar Pradesh, West Bengal, Rajasthan, Gujraat, Haryana, Maharashtra, Chhatisgarh and even in Tamil Nadu. Quality fruits with longer shelf life are the requirement of processing industry. The foliar application of calcium chloride and GA_3 have very important role in increasing the shelf life of fruits. Earlier research shows that foliar spraying of micronutrients and plant growth regulators increased the quality and shelf life of the fruits, Singh and Vashistha (1999) and Srivastava (2003).

Calcium compound have shown promise in the quality retention of fruits (Huber, 1983). The present investigation was conducted to find out the effectiveness of pre-harvest sprays of CaCl_2 , GA_3 and their combinations on shelf life of aonla fruits at ambient storage.

Materials and Methods

The experiment was carried out on seventeen years old trees of aonla cv. Chakaiya planted under sodic soil conditions at Main Experiment Station of Horticulture Department, N.D. University of Agriculture & Technology, Kumarganj, Faizabad, Uttar Pradesh, during 2009-10. The experiment consists of nine treatments including calcium chloride, GA_3 & its different combinations as well as control. The spray solutions were prepared in water and few drops of alcohol were used to dissolve the GA_3 before mixing it in water and hair shampoo was used as sticker. The spraying was done on the randomly selected bearing trees by sprayer 25 days before harvesting the fruits. The water with sticker was sprayed on control. The fruit were harvesting at full maturity and stored in plastic baskets at ambient temperature ($7.48-21.34^\circ\text{C}$) for storage studies. Two kg fruits from each replication of every treatment were harvested and mixed together thus 6 kg fruits were used in each treatment for storage studies. The observations were recorded at an interval of five days during storage. The experiment was laid out in complete randomized design with three replications. The physiological loss in weight was recorded on physical balance. The fruits started decaying

was isolated from the lot and their weight was recorded. The per cent decay loss was calculated against the initial weight of the lot. Total soluble solids was estimated with ERMA hand refractometer of 0-32% range and calibrated at 20°C whereas to determine acidity a known volume of sample was titrated against 0.1 N NaOH solutions using phenolphthalein as an indicator. Vitamin 'C' was estimated by titration of a known volume of sample in 3% metaphosphoric acid against 2, 6 dichlorophenol indophenols dye (A.O.A.C., 1970). Total phenols were extracted into 80% ethyl alcohol and colour was developed by adding phenol reagent and sodium carbonate solution. The colour was measured at 750 nm using spectrophotometer. The total phenols were calculated by the standard curve drawn with graded concentration of Gallic acid (Swan and Hills, 1959). The reducing, non-reducing and total sugars were estimated using Fehling's solutions method given by Lane and Eynon (1923) as described by Ranganna (1978).

Results and discussion

The physiological loss in weight among treatments significantly increased with the storage period. CaCl_2 (2%) + GA_3 (100 ppm) sprayed fruits showed lowest weight loss (16.28%), while fruits under control recorded the highest loss in weight (25.56%) at 20th day of storage (Table-1). This may be due to the role of calcium on limiting respiration which was attributed to altered membrane permeability (Bangerth, 1979). Bangerth *et al.* (1972) also reported that calcium could have reduced the endogenous substrate catabolism during respiration by limiting the diffusion of substrate from the vacuole to the cytoplasm and favoured the uptake of sorbitol, thus, disallowing its involvement in reactions related to internal breakdown. Similar results on reduction in weight loss by calcium chloride and GA_3 treatments were also reported in ber by Gupta *et al.* (1987) and in peaches by Bhullar *et al.* (1982).

The total soluble solids were significantly increased among all treatments with the storage period (Table-2). CaCl_2 (2%) + GA_3 (100 ppm) treated fruits had maximum T.S.S. content throughout the storage period in comparison to other treatments. Higher total soluble solids content in CaCl_2 (2%) + GA_3 (100 ppm) treated fruit was attributed to increase in dry matter accumulation particularly starch, which in turn broken down to simple sugars during maturation and ripening. (Upadhya and Dixit, 1996) and Kumar *et al.* (2005) have also reported that CaCl_2 and GA_3 increased the T.S.S in aonla fruits which support the present finding.

The acidity was significantly influenced by CaCl_2 (2%) and GA_3 (100 ppm) there was an initial increase in acidity up to 5 days of storage and thereafter decreasing trend was observed till the end of storage (Table-3). An increase in acidity of initial stage might be due to biosynthesis of citric acid initially. Latter on decrease in acidity may be due to conversion of acids into sugars (Pool

et al., 1972). Similarly CaCl_2 and GA_3 have been found to increase and maintained higher acidity in guava Kher *et al.* (2004). The fruits pre harvest sprayed with CaCl_2 (2%) and GA_3 (100 ppm) retained the maximum ascorbic acid (725.9 mg/100g) than other treatments on the date of harvest (Table-4). Pre harvest spray of CaCl_2 + GA_3 has increased the ascorbic acid that might be due to increased level of calcium in the fruits. The ascorbic content slowly decreasing in all the treatments without any significant change in the rate of decrease but retention was higher in CaCl_2 (2%) and GA_3 (100 ppm) treated fruits during storage in comparison to other treatments. The loss in ascorbic acid is due to rapid conversion of L-ascorbic acid into dehydro ascorbic acid in the presence of ascorbinase enzyme (Mapson, 1970) similar results on retention of ascorbic acid by CaCl_2 and GA_3 treatment were also reported in mango (Mukherjee and Srivastava, 1978).

The level of reducing sugar was higher on the date of harvest in fruits which were given pre-harvest treatments of CaCl_2 and GA_3 and their different combinations than control however maximum reducing sugar was recorded in CaCl_2 (2%) and GA_3 (100 ppm) treated fruits. This clearly indicates the treatment effect of reducing sugar content. Initially the reducing sugar was slightly increased up to 5 days (Table-5) but starts decreasing after 10 days of storage. The fruits treated with CaCl_2 (2%) and GA_3 (100 ppm) retained higher reducing sugar than other treatments. The initial rise in sugar content might be due to conversion of starch into sugars, while the subsequent decline was perhaps due to consumption of sugar for respiration. CaCl_2 and GA_3 are reported to protect reducing sugar in strawberry (Singh, *et al.* 2004).

Non-reducing sugar content of fruits did not differed significantly at harvest. However it was increased in all the treatments during storage upto 15 days thereafter started decreasing (Table-6). Pre-harvest spray of CaCl_2 (2%) and GA_3 (100 ppm) maintained highest level of non-reducing sugar followed by CaCl_2 (1%) and GA_3 (200 ppm) in comparison to control. The decreasing in non-reducing sugar in control was faster than in treated fruits. Calcium and GA_3 have also been reported to protect non-reducing sugar in litchi (Upreti and Kumar, 1996).

Similarly total sugar content was significantly highest in CaCl_2 (2%) and GA_3 (100 ppm) treated fruits whereas fruits get other treatments also contains higher total sugar in comparison to control. Total sugars content initially increased but started decreasing after 15 days in all the treatments that was continued till the end of storage. (Table-7). It clearly showed the effect of treatments on level of total sugar content during storage of aonla fruits. Calcium and GA_3 are reported to protect total sugar in litchi (Rani and Brahmachari, 2002).

The total phenols were significantly higher in all treatments than control on the day fruit harvested and this difference continued up to 25th day of storage. Highest total phenol content was in fruit treated with CaCl_2 (2%) and GA_3 (100 ppm) in comparison to other treatments and was

lowest in control. Total phenol increased till end of storage (Table-8). Increasing in total phenol might be due to loss of moisture and increase in dry matter accumulation. A total phenol is reported to increase during storage of aonla fruits by other scientists like Singh *et al.* (1993) and Singh and Kumar (2000).

The decay loss among treatments significantly increased with the storage period but CaCl_2 (2%) and GA_3 (100 ppm) treated fruits recorded the lowest decay loss while control recorded the highest decay loss (Table-9). There was no decay loss upto 5 days in all treatments. However, in (CaCl_2 2% and GA_3 100 ppm) treated fruits decay loss was 15.62% after 20 days yet it was lowest among all treatments. The CaCl_2 and GA_3 appear to be enhancing shelf life by controlling decay loss due to increased Ca and GA_3 level in fruits. The decay loss by CaCl_2 application has also been controlled in grape (Ferguson *et al.*, 1983).

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Table 1. Pre-harvest treatments effect on PLW (%) during ambient storage of aonla fruits.

Treatments	Days of storage				
	5	10	15	20	25
T ₁ - Control (water)	3.33	9.05	17.61	25.56	31.48
T ₂ -100 ppm GA ₃	3.04	7.58	13.76	20.46	25.71
T ₃ -1% CaCl ₂	2.42	6.75	12.51	18.45	23.62
T ₄ -1% CaCl ₂ +100 ppm GA ₃	2.17	7.15	12.66	19.37	21.67
T ₅ -1.5% CaCl ₂ +100 ppm GA ₃	1.92	6.22	11.10	18.49	22.16
T ₆ -2% CaCl ₂ +100 ppm GA ₃	1.61	5.03	9.79	16.28	20.41
T ₇ -50 ppm GA ₃ +1% CaCl ₂	2.66	8.23	13.29	18.40	22.30
T ₈ -150 ppm GA ₃ +1% CaCl ₂	2.15	7.33	13.04	18.08	21.46
T ₉ -200 ppm GA ₃ +1% CaCl ₂	2.12	6.69	10.06	17.53	21.53
CD at 5%	NS	3.44	6.95	7.88	10.11

Table 2. Pre-harvest treatments effect on total soluble solids (%) during ambient storage of aonla fruits.

Treatments	Days of storage					
	0	5	10	15	20	25
T ₁ - Control (water)	8.73	8.97	9.37	9.73	10.07	10.37
T ₂ -100 ppm GA ₃	8.77	9.03	9.67	10.43	11.23	11.33
T ₃ -1% CaCl ₂	8.80	9.67	10.27	10.83	11.47	11.63
T ₄ -1% CaCl ₂ +100 ppm GA ₃	8.83	9.70	10.47	10.70	11.63	11.73
T ₅ -1.5% CaCl ₂ +100 ppm GA ₃	9.51	10.10	10.57	11.23	11.73	12.00
T ₆ -2% CaCl ₂ +100 ppm GA ₃	10.20	10.67	11.43	11.90	12.57	12.63
T ₇ -50 ppm GA ₃ +1% CaCl ₂	8.97	10.07	10.73	11.30	11.77	11.90
T ₈ -150 ppm GA ₃ +1% CaCl ₂	9.23	10.50	10.70	11.77	12.03	12.37
T ₉ -200 ppm GA ₃ +1% CaCl ₂	9.70	10.57	11.13	11.83	12.23	12.53
CD at 5%	0.25	0.26	0.21	0.21	0.27	0.31

Table 3. Pre-harvest treatments effect on acidity (%) during ambient storage of aonla fruits.

Treatments	Days of storage					
	0	5	10	15	20	25
T ₁ - Control (water)	1.88	2.17	1.75	1.53	1.37	1.24
T ₂ -100 ppm GA ₃	2.09	2.30	1.83	1.57	1.51	1.37
T ₃ -1% CaCl ₂	2.22	2.61	1.88	1.62	1.53	1.45
T ₄ -1% CaCl ₂ +100 ppm GA ₃	2.39	2.68	1.92	1.73	1.62	1.53
T ₅ -1.5% CaCl ₂ +100 ppm GA ₃	2.56	2.72	2.13	1.74	1.65	1.57
T ₆ -2% CaCl ₂ +100 ppm GA ₃	2.85	2.94	2.30	1.88	1.75	1.66
T ₇ -50 ppm GA ₃ +1% CaCl ₂	2.26	2.60	1.83	1.70	1.61	1.51
T ₈ -150 ppm GA ₃ +1% CaCl ₂	2.43	2.68	1.88	1.72	1.62	1.53
T ₉ -200 ppm GA ₃ +1% CaCl ₂	2.72	2.85	1.92	1.75	1.67	1.57
CD at 5%	0.15	0.18	0.35	0.15	0.17	0.17

Table 4. Pre-harvest treatments effect on ascorbic acid (mg/100 g) during ambient storage of aonla fruits.

Treatments	Days of storage					
	0	5	10	15	20	25
T ₁ - Control (water)	663.67	636.73	577.37	508.40	448.07	421.37
T ₂ -100 ppm GA ₃	697.63	675.80	631.17	579.60	492.47	465.50
T ₃ -1% CaCl ₂	706.36	685.03	661.67	590.50	530.37	479.03
T ₄ -1% CaCl ₂ +100 ppm GA ₃	710.70	695.33	668.73	603.67	544.33	486.93
T ₅ -1.5% CaCl ₂ +100 ppm GA ₃	717.93	699.60	676.20	618.23	559.23	499.60
T ₆ -2% CaCl ₂ +100 ppm GA ₃	725.90	707.63	682.60	631.60	572.13	538.00
T ₇ -50 ppm GA ₃ +1% CaCl ₂	709.27	688.10	660.20	601.23	542.50	480.63
T ₈ -150 ppm GA ₃ +1% CaCl ₂	715.73	698.43	670.87	617.10	554.33	495.80
T ₉ -200 ppm GA ₃ +1% CaCl ₂	720.10	705.41	678.33	624.33	566.57	514.70
CD at 5%	3.69	3.94	6.84	5.89	8.13	32.98

Table 5. Pre-harvest treatments effect on reducing sugar (%) during ambient storage of aonla fruits.

Treatments	Days of storage					
	0	5	10	15	20	25
T ₁ - Control (water)	2.10	2.21	1.97	1.91	1.73	1.57
T ₂ -100 ppm GA ₃	2.45	2.57	2.30	1.99	1.90	1.70
T ₃ -1% CaCl ₂	2.64	2.73	2.37	2.06	1.91	1.80
T ₄ -1% CaCl ₂ +100 ppm GA ₃	2.87	3.11	2.57	2.31	2.03	1.82
T ₅ -1.5% CaCl ₂ +100 ppm GA ₃	2.91	3.19	2.70	2.32	2.15	1.87
T ₆ -2% CaCl ₂ +100 ppm GA ₃	2.97	3.28	2.87	2.43	2.41	2.17
T ₇ -50 ppm GA ₃ +1% CaCl ₂	2.61	2.91	2.57	2.11	2.00	1.80
T ₈ -150 ppm GA ₃ +1% CaCl ₂	2.67	3.11	2.62	2.23	2.25	1.91
T ₉ -200 ppm GA ₃ +1% CaCl ₂	2.94	3.24	2.73	2.37	2.33	2.10
CD at 5%	0.42	0.20	0.22	0.22	0.11	0.28

Table 6. Pre-harvest treatments effect on non reducing sugar (%) during ambient storage of aonla fruits.

Treatments	Days of storage					
	0	5	10	15	20	25
T ₁ - Control (water)	2.03	2.34	2.64	2.72	2.56	2.37
T ₂ -100 ppm GA ₃	2.32	2.53	2.93	3.11	2.92	2.87
T ₃ -1% CaCl ₂	2.34	2.55	3.12	3.27	2.89	2.83
T ₄ -1% CaCl ₂ +100 ppm GA ₃	2.38	2.57	3.10	3.44	3.02	2.99
T ₅ -1.5% CaCl ₂ +100 ppm GA ₃	2.43	2.60	3.15	3.55	3.22	3.15
T ₆ -2% CaCl ₂ +100 ppm GA ₃	2.48	2.62	3.27	3.78	3.31	3.22
T ₇ -50 ppm GA ₃ +1% CaCl ₂	2.37	2.57	2.99	3.35	3.15	3.10
T ₈ -150 ppm GA ₃ +1% CaCl ₂	2.41	2.59	3.18	3.62	3.21	3.12
T ₉ -200 ppm GA ₃ +1% CaCl ₂	2.45	2.61	3.21	3.76	3.28	3.23
CD at 5%	0.32	0.19	0.27	0.36	1.16	0.46

Table 7. Pre-harvest treatments effect on total sugar (%) during ambient storage of aonla fruits.

Treatments	Days of storage					
	0	5	10	15	20	25
T ₁ -Control (water)	4.13	4.55	4.61	4.63	4.29	3.94
T ₂ -100 ppm GA ₃	4.77	5.18	5.23	5.17	4.82	4.57
T ₃ -1% CaCl ₂	4.98	5.35	5.49	5.38	4.89	4.63
T ₄ -1% CaCl ₂ +100 ppm GA ₃	5.17	5.19	5.67	5.75	5.05	4.79
T ₅ -1.5% CaCl ₂ +100 ppm GA ₃	5.34	5.75	5.85	5.87	5.37	5.02
T ₆ -2% CaCl ₂ +100 ppm GA ₃	5.45	5.89	6.14	6.17	5.72	5.39
T ₇ -50 ppm GA ₃ +1% CaCl ₂	4.98	5.48	5.56	5.34	5.05	4.90
T ₈ -150 ppm GA ₃ +1% CaCl ₂	5.08	5.68	5.83	5.85	5.46	5.03
T ₉ -200 ppm GA ₃ +1% CaCl ₂	5.39	5.83	5.94	6.13	5.61	5.33
CD at 5%	0.46	0.59	0.49	0.59	0.26	0.74

Table 8. Pre-harvest treatments effect on total phenol (mg/100g) during ambient storage of aonla fruits.

Treatments	Days of storage					
	0	5	10	15	20	25
T ₁ -Control (water)	146.33	152.00	159.67	165.67	173.33	182.33
T ₂ -100 ppm GA ₃	153.00	161.33	168.67	175.33	181.67	185.00
T ₃ -1% CaCl ₂	154.67	164.00	171.00	176.33	183.33	190.67
T ₄ -1% CaCl ₂ +100 ppm GA ₃	160.33	167.67	174.00	179.67	187.33	195.00
T ₅ -1.5% CaCl ₂ +100 ppm GA ₃	164.00	170.37	176.00	182.00	190.67	197.33
T ₆ -2% CaCl ₂ +100 ppm GA ₃	170.67	178.00	186.67	195.67	203.00	210.67
T ₇ -50 ppm GA ₃ +1% CaCl ₂	159.00	165.00	172.33	176.33	185.33	192.33
T ₈ -150 ppm GA ₃ +1% CaCl ₂	162.33	168.33	175.00	180.33	187.33	193.33
T ₉ -200 ppm GA ₃ +1% CaCl ₂	167.67	176.33	182.00	190.67	197.67	206.33
CD at 5%	1.51	1.55	1.84	1.36	1.68	2.78

Table 9. Pre-harvest treatments effect on decay loss (%) during ambient storage of aonla fruits.

Treatments	Days of storage				
	5	10	15	20	25
T ₁ -Control (water)	00	6.29 (2.61)	15.03	27.05	41.31
T ₂ -100 ppm GA ₃	00	5.02 (2.35)	12.75	20.50	32.25
T ₃ -1% CaCl ₂	00	3.04 (1.88)	11.41	19.88	28.58
T ₄ -1% CaCl ₂ +100 ppm GA ₃	00	00 (0.71)	8.04	17.59	26.38
T ₅ -1.5% CaCl ₂ +100 ppm GA ₃	00	00 (0.71)	6.85	16.66	25.47
T ₆ -2% CaCl ₂ +100 ppm GA ₃	00	00 (0.71)	4.21	15.62	22.89
T ₇ -50 ppm GA ₃ +1% CaCl ₂	00	3.57 (2.02)	11.58	18.92	27.77
T ₈ -150 ppm GA ₃ +1% CaCl ₂	00	00 (0.71)	7.14	17.91	25.20
T ₉ -200 ppm GA ₃ +1% CaCl ₂	00	00 (0.71)	6.07	15.67	24.54
CD at 5%	-	0.03	1.73	1.93	1.73

Note- Transformed data ($Y = \sqrt{x + \frac{1}{2}}$) are shown in parenthesis.

Assessment of population of pseudomonads inhabitant of tomato plant rhizosphere for searching of potential antagonist of *Fusarium udum*

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Abstract

Eight isolates of fluorescent pseudomonads were isolated from the disease suppressed and identified and characterized by physiological and biochemical and bacteriological tests. LPK2 aligned to same cluster (100% bootstrap value) with reference strains of *Pseudomonas fluorescens*. All the strains of *P. fluorescens* LPK2 showed IAA production, phosphate solubilisation, siderophore production and HCN production. Chitinase activity and β -1,3-glucanase activity was also pronounced in *P. fluorescens* LPK2. In dual culture technique, all the strains of *Pseudomonas* (LPK1 to LPK8) inhibited the *in vitro* growth of *F. udum*. Maximum inhibition of *F. udum* recorded in the case of *P. fluorescens* LPK2. Cell free culture filtrate of also restricted the hyphal growth of *F. udum*. Scanning electron microscopic studies on morphological features in the hyphae of *F. udum* in the form of fragmentation and degradation of mycelia and loss of structural integrity of conidia of *F. udum* were clearly observed. In iron supplemented (30 μ M Fe³⁺) medium inhibition of the pathogenic fungi was reduced. Under iron-deficient condition, *P. fluorescens* LPK2 inhibited *F. udum* by 65%. The inhibition of the pathogen by *P. fluorescens* LPK2 under iron-sufficient conditions was 51%.

Key words : *Fusarium udum*, pseudomonads, tomato.

Introduction

India is a principal *Cajanus cajan* growing country contributing nearly 90% of total world's production. Several biotic and abiotic factors constrain the productivity of pigeonpea under different cropping conditions responsible for its low productivity (700-800 kg/ha) against potential yield of 2500-3000 kg/ha. Pigeonpea, is attacked by more than 100 pathogens (Nene *et al.*, 1996). The disease of considerable economic importance at present is fusarium wilt (*Fusarium udum*). It infests the seeds and such seeds either failed to germinate or produce diseased seedlings of low survival potential.

The chemical fungicides and pesticides are known to be highly effective and promote in controlling fusarium wilt but have various constraints. An alternative to these chemicals is the use of certain bio-control agents which are inexpensive and eco-friendly and have no harmful effects on human population. The plant-associated, fluorescent

pseudomonads have drawn considerable attention world wide because of their plant growth promoting and disease suppressive nature. Pseudomonads rapidly and aggressively colonize the root system and suppress pathogenic microorganisms improving plant growth and grain yield (Schippers *et al.*, 1987; Weller, 1988). In this study, the main aim is to investigate the potential fluorescent pseudomonads and their role in suppression of *F. udum* causing wilt in *C. cajan*.

Materials and methods

Fluorescent bacteria were isolated from the disease suppressed rhizosphere of the tomato (*Lycopersicon esculentum*) plant cultivated at a farmer's field in Badgaon, Betul district (M.P.), India. Plants were gently uprooted and loosely adhering soil was carefully removed from the roots. The roots were cut into 2 cm long segments. Root segments (1g) with tightly adhering soil were shaken in 9 ml of sterile distilled water for 30 minutes on a rotary shaker. The suspension was serially diluted to spread on *Pseudomonas* isolation agar (Himedia; M406) plates. Plates

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were incubated at $28 \pm 1^\circ\text{C}$ for 24-48 h and observed for fluorescent colonies. The blue green fluorescent pigment producing bacterial colonies were carefully picked up and maintained on King's B medium (KBM) slants at 4°C for further screening. Biochemical characterization of the bacteria strains was carried out according to Holt *et al.*, 1994.

Isolation and identification of fungal pathogen

Samples of diseased root parts and seeds were randomly collected and fungal pathogen was isolated from infected roots of pigeon pea (*Cajanus cajan*) following the water agar technique. After incubation at 28°C for one week, plates were observed for the appearance of fungal pathogen. The fungal isolates were grown on the potato dextrose agar (PDA) medium and cultures were maintained on potato dextrose agar (PDA) medium and characterized following illustration and description of standard mycological literature after comparing with standard cultures obtained from the Division of Plant Pathology, Indian Institute of Pulses Research (IIPR), Kanpur, India. The morphological characters of *F. udum* such as colony growth, colour pigmentation in substrate, mycelial characters, and micro and macro conidia production were considered for the identification of fungi as given by Booth (1971).

IAA Production

To observe IAA production exponentially grown cultures (10^8 cells ml^{-1}) of the each bacterial isolate were strains were incubated separately. The 24 h cell free culture supernatants of the strains were analyzed for IAA production after measuring wavelength at 530 nm using UV-VIS spectrophotometer (Shimadzu, Model UV-1601. The calibration curve of pure IAA was used as a standard following the linear regression analysis.

Phosphate Solubilization

Phosphate solubilization ability was detected by spotting the culture (24 h) on Pikovskya's agar plates. The quantitative estimation of phosphate solubilization was carried out according to modified method of Jackson (1958). The data was recorded by comparing with the standard curve in the similar set of conditions.

Cyanogen Determination

HCN (hydrogen cyanide) production was determined by modified method of Bakker and Schippers (1987). For these exponentially grown bacterial cultures (10^8 cells ml^{-1}) of each isolate was examined for putative HCN production.

Effect of HCN on fungal growth: The strains of fluorescent *Pseudomonas* were grown on Petri plates containing NAM for 12 h. An agar discs (5 mm diam) cut from 5 day old cultures of *F. udum* was placed at the center

of bottom part of another plate containing PDA. The plates inoculated with fungi were placed in an inverted position over the other plate, containing already grown culture of the fluorescent *Pseudomonas*. The plates were made airtight by pasting with adhesive cellophane tape. The plates with NAM without fluorescent *Pseudomonas* served as control. Radial growth of the test fungi was recorded.

Siderophore Production

Siderophore production was determined on Chrome-azurol S (CAS) medium following the method of Schwyn and Neilands (1987). The bacterial isolates (24 h) spotted separately on CAS medium and incubated at $28 \pm 1^\circ\text{C}$ for 48 h. The siderophores production was determined by evaluating the absorption spectrum of 48 h supernatant at 400 nm, spectrophotometrically.

Siderophore Assay: For siderophore production by bacteria, a special siderophore production liquid culture was prepared which contained MM9 salts, tris buffer, casamino acids (0.3%), L-glutamic acid (0.05%), (+) biotin (0.5 ppm) and sucrose (0.2%). Bacterial cultures were grown in this medium for 48 h at 28°C under rotating conditions and its cell density was monitored spectrophotometrically at 610 nm. After incubation, culture supernatant (0.5 ml) was mixed with 0.5 ml of CAS assay solution. An uninoculated medium served as reference. After reaching colour equilibrium the absorbance of the mixture was measured at 630 nm. The concentration of siderophore in cultures was determined by using a calibration curve of hydroxylamine as a standard under the same conditions and following the linear regression analysis.

Time course of siderophore production: Following the above method of quantification of siderophore production, samples from the bacterial broth were withdrawn at every 24 h intervals up to 168 h for the quantitative determination of siderophore. A plot was prepared comparing siderophore production with respect to time.

Lytic Enzymes Production

Chitinase activity was observed by the method of Renwick *et al.* (1991) in a defined medium having colloidal chitin as sole carbon source. The bacterial cultures were spotted on the surface of defined medium and incubated at $28 \pm 1^\circ\text{C}$ for 6-7 days. β -1,3-glucanase activity was assessed using the same medium except colloidal chitin was replaced by laminarin (Sigma). Bacterial cultures were streaked and/or spot inoculated directly on to the plates. Chitinase activity was identified by the development of clear halo around the colonies or bacterial spots, while β -1,3-glucanase activity was confirmed with the bacteria growing on the medium having laminarin as a sole source of carbon (Rangel-Castro *et al.*, 2002).

For the preparation of colloidal chitin (Berger and Reynoldes, 1988), 10 g of crab shell chitin (Sigma) was slowly dissolved in one liter of concentrated HCl under stirring

conditions at 4°C temperature. The resultant viscous mixture was incubated on waterbath at 37°C until the viscosity of the mixture is decreased. To this mixture, 4 liters of sterile distilled water was added and left overnight at 4°C. The supernatant was slowly decanted and the precipitate was collected in a filter paper. This precipitate was then washed extensively with sterile distilled water to attain a natural pH (7.0). The saturated colloidal chitin was air dried and was dissolved in 250 ml sterile distilled water prior to use.

Biochemical quantification of chitinase:

Extracellular chitinase activity was assayed by measuring the release of N-acetyl-D-glucosamine (Glc Nac) from colloidal chitin as a substrate. The assay mixture consisted of 0.3 ml of 1 M sodium acetate buffer (pH 5.3), 0.5 ml of 2% colloidal chitin and 0.25 ml of bacterial supernatant. The reaction mixture was incubated in a water bath at 37°C for 60 min and the reaction was stopped after boiling it for 15 min. The mixture was centrifuged at 5,000 rpm for 20 min and the concentration of released Glc Nac was assayed at 530 nm spectrophotometrically from the aliquots following DNS sugar estimation test using Glc Nac as standard. One unit of chitinase activity was defined as the amount of enzyme that liberated 1 µM of Glc Nac per h during these conditions.

Production profile of chitinase in *P. fluorescens*

LPK2: Exponentially grown culture (10^8 cells ml⁻¹) of strains were incubated separately on broth medium at 28 °C and 150 rpm on a rotary incubator shaker and the samples were aseptically withdrawn after every 24 h. Supernatant of the strains were collected by centrifugation at 10,000 rpm for 15 min at 4°C and quantity of chitinase in the supernatant in the form of Glc Nac was determined following above mentioned method, and the production profile was observed by correlating the quantity of Glc Nac with time.

DNA isolation

For the isolation of DNA from fluorescent *Pseudomonas* spp. were grown in Luria Bertani (LB) broth. The bacterial pellets were washed with 50 mM ethylenediamine tetraacetic acid (EDTA, pH 8.5). The washed cells were lysed by suspending in 0.5% sodium dodecyl sulphate (SDS) for 10 min at 60°C. The resulting lysate was cleared from cell debris by centrifugation at 10,000 rpm for 10 min. The supernatant was taken and protein was removed by the addition of proteinase K followed by addition of ethanol to collect the DNA. The DNA solution was prepared by the addition of 1 ml phenol-chloroform mixture. To the DNA extract 50 µg ml⁻¹ RNase was added and incubated at 37°C for 30 min. This was followed by the addition of tris-phenol and centrifugation at 12,000 rpm for 15-20 min at 4°C. The supernatant was heat sterilized and 100 µl, 0.3 M sodium acetate and 1 ml cold isopropanol was added with continuous vortex. Finally, the DNA was purified by the addition of phenol-chloroform followed by the centrifugation at 15000 rpm.

16S rRNA Gene Amplification

Universal eubacterial primers FD1 5'CCGAATTCGTCGACAACAGAGTTTGATCCTGGCTCAG3' and RD1 5'CCCGGGATCCAAGCTTAAGGAGGTGATCCAGCC3' were used for amplification of 1492 bp region of the 16S rRNA gene on PTC 100 (M.J. Research, USA) thermal cycler. A 50 µl reaction mixture included 5-10 ng of bacterial DNA as template, 1 µl of each primer, 1U of *Taq* DNA polymerase (Bangalore Genei, India) and 100 µM dNTPs. The reaction conditions were: initial denaturation of 7 min at 94°C followed by 29 cycles of denaturation of 1 min at 94°C, extension of 1 min at 72°C and annealing temperatures 54°C for 7 cycles; 53°C and 52°C for one cycle each, 51°C for 20 cycles and a final extension of 10 min at 72°C. Amplified gene was visualized in 0.8% agarose after electrophoresis.

16s rRNA gene sequencing:

Partial 16S rRNA gene sequencing was performed in the same reaction mixture and following the same amplification conditions as described in full 16S rDNA. The only difference was in primers. In the partial gene amplification, primers fl and r1 were used. The PCR products were analyzed on 1.2 % agarose gel in TAE buffer, run at 50 V for 2 h. Gels were stained with ethidium bromide and visualized as described above. The amplicons were purified with Bangalore Genei, PCR purification kit and quantified spectrophotometrically at 260 nm compare with calf thymus DNA. The cleaned partial 16S rDNA amplicon was sequenced with DNA sequencing system.

Sequence analysis of 16S rDNA sequences using bioinformatics tools:

The PCR products were purified and sequenced. These all sequences were subjected for phylogenetic analysis. The homology of partial sequences were compared with the sequences from the DNA databases and similar sequences showing above 95% were retrieved by nucleotide BLAST (basic local alignment search tool) program at NCBI BLAST server (www.ncbi.nlm.nih.gov/BLAST). Multiple sequence alignment of retrieved sequences was done by EBI ClustalW server (www.ebi.ac.uk/clustal/index.html). Phylogenetic tree constructed by using *genebee* server (www.genebee.msu.ru/services/phtree-reduced.html). Phylogenetic tree obtained with bootstrap values in cluster algorithm, phylip format and topological algorithm

Antifungal activities of *Pseudomonas fluorescens* LPK2

Antagonistic properties of *Pseudomonas fluorescens* LPK2 was tested against *F. udum* on PDA plates using dual culture technique (Skidmore and Dickinson, 1976). Five days old mycelial discs (5 mm diameter) were placed in four corners of solidified medium in plates containing modified PDA by addition of 2% sucrose.

Exponentially grown culture (108 ml-1) of LI 32 was spotted 2 cm juxtaposed from the fungal disc and incubated at $28 \pm 10^\circ\text{C}$ for 5 days. Growth inhibition was calculated by measuring the distance between the bacterial and fungal colonies as compared to control. Growth inhibition was calculated by measuring the distance between the edge of bacterial and fungal colonies. The zone of inhibition was recorded by using given below formula: Inhibition (%) = $(C) - (T) / (C) \times 100$, where, C=Radial growth in control and T=Radial growth in dual culture.

Effect of culture filtrates on the inhibition of pathogenic fungi

Antagonistic bacteria were inoculated in their respective broth cultures and incubated in rotary incubator shaker at 150 rpm and 280°C for 7 days. After incubation broth culture of the strains were collected by centrifugation at 10,000 rpm for 15 min at 40°C and passed through Millipore filter (0.45 μm). Such culture supernatants were poured in the wells of the fungal challenged plates. After incubation at 280°C for 5-7 days, inhibition of the pathogenic fungi was recorded.

Antagonism in iron-deficient and iron sufficient conditions

In vitro test for antagonism of *P. fluorescens* LPK2 was made under iron-deficient and iron-sufficient (30 and 60 μM Fe^{3+}) conditions towards the *Fusarium udum*. Iron deficient medium was prepared by treating bacterial broth (250 ml) with 8-hydroxyquinoline solution in 100 ml chloroform (up to final concentration (0.1%). It was then vigorously shaken. Excess of hydroxyquinoline was removed by chloroform extraction until the disappearance of yellow color. Medium was boiled to remove the smell of chloroform. Agar (2%) was added and the content autoclaved at 15 lb /inch-2 (1210°C) for 20 min. Antagonism was carried out by following the methodology mentioned as before. Bacterial broth supplemented with 30 and 60 μM Fe^{3+} was used to carry out *in vitro* fungal assay under iron sufficient condition. Methodology for antagonism study was adopted as mentioned above.

Compound microscopy of post-interaction events in fungal mycelia

Fungal mycelia growing towards the zone of inhibition were processed for microscopy. Fungal mycelium were picked up from the zone of inhibition with the help of sterile needle and transferred to a drop of lacto-phenol on the clean glass slide. Specimens were examined under a light compound microscope (Olympus BX 51 TRF) for fungal morphological abnormalities occurred due to antagonism mediated by the rhizobial strains. Images of fungal deformities were captured by using Image Analyser (Biovis).

Scanning electron microscopy (SEM) of post-interaction events in fungal mycelia

The antagonist and the test organism are grown in pure cultures. Agar disc (5mm diameter) are to be cut from the actively growing margin of the colonies and placed 3cm apart on the surface of the Petri dishes containing freshly prepared, sterilized and solidified potato Dextrose agar medium. Incubated Petri dishes at 25°C with continuous light after incubation of 4 days collect mycelial sample from the region of interaction. For preparation of SEM samples, the mycelia were collected with the help of micro-needle in sterile environment from the zone of interaction with bacteria on the surface of gelled agar in Petri plate. Then it were fixed overnight at 4°C in 4% glutaraldehyde in 0.05 M phosphate buffer (pH 7.3) and washed three times (10 min each) in phosphate buffer. After three 10 min rinses in distilled water, samples were dehydrated through 70, 80, 90 and 100% ethanol (5 min in each stage) and three changes in 100% ethanol at room temperature. Ethanol was then replaced by liquid CO_2 and the samples were air dried. Then samples were mounted on stubs and coated with gold. These coated specimens were observed at 15 kV in a LEO 485 VP Scanning Electron Microscope. Photo-micrographs were recorded by the same machine.

Results

Mycelia were hyaline, branched and felted. Aerial mycelia were usually less developed and with profuse development of sporodochia (macroconidiophores), long creeping, slender, septate and measured 3-5 μm in width on PDA. Conidia were formed from simple or verticillately branched conidiophores and each conidiophore had monophialides on the ultimate tips of which conidia were produced. Microconidia were single or double celled hyaline, ovoid, fusoid, reniform, mostly curved and scattered, whereas macroconidia were produced in masses on pionnotes sporodochia. Macroconidia were hyaline, thin walled, falcate, septate slightly curved or sickle shape.

Eight isolates of fluorescent pseudomonads were obtained from the disease suppressed rhizosphere of the tomato (*Lycopersicon esculentum*) based on production of water soluble fluorescent pigment on *Pseudomonas* isolation agar (PIA). The isolates were abbreviates as LPK1 to LPK8. All the isolates were Gram-negative, non-spore forming, non-capsulated, motile and rod shape structures. They were fast growers with average mean generation time of 1.2 to 1.4 hours (Table 1). Although the optimum temperature was 28°C but growth occurred at wide range of temperatures from 5°C to 42°C . On NAM the colonies were smooth, translucent, large, low convex, 2-4 mm in diameter with regular spreading edge. Greenish blue fluorescent pigment was produced by the isolates which turned into brown colour in old cultures. On MacConkey's agar, pale colourless colonies were formed. The isolates were positive

for catalase, oxidase, and urease activities but negative for MR, VP tests and H_2S production. PHB accumulation was absent while citrate utilization was shown by all the fluorescent pseudomonad isolates (Table 1).

After amplification of 16S rRNA gene with universal fd1 and rd1 primers, the amplified products were compared with low range ruler DNA (Bangalore Genei, India) and observed that total length of amplicons was approximately 1500 bp (1.5 kb) which shows the amplification of nearly full length 16S rRNA gene from all pseudomonads. The size of the PCR product corresponded to the size of the 16S rRNA genes among all eight isolates.

Neighbour-joining dendograms were generated (based on cluster algorithm) using 16S rDNA sequences of isolated pseudomonads and representative *Pseudomonas* spp. sequences from EMBL/Gen Bank/DDBJ/PDB (Fig. 1). The 16S rDNA sequence of the following strains (type strains unless otherwise indicated) were obtained from GenBank: *Pseudomonas aeruginosa* PT121 (EF515832), *Pseudomonas aeruginosa* PAW (EF434506), *Pseudomonas fluorescens* 2R37 (EF178447), *Pseudomonas fluorescens* ost5 (DQ439976), *Pseudomonas fluorescens* AE1 (AY247063), *Pseudomonas aeruginosa* ATCC 15692 (EF178445), *Pseudomonas fluorescens* ATCC 17386, *Pseudomonas fluorescens* MM-B16, *Pseudomonas fluorescens* AU2039A, *Pseudomonas aeruginosa* MCCB103 *Pseudomonas aeruginosa* PT121 (EF515832). Phylogenetic analysis of 16S rDNA sequences of pseudomonads (LPK1 to LPK5) with other reference strains revealed LPK1 and LPK3 belong to same cluster with 100% bootstrap value and maximum similarity with LPK4 (100%), *P. aeruginosa* PT121 (94%) and *P. aeruginosa* PAW (94%) (Fig. 1). LPK2 and LPK5 showed maximum similarity with *P. fluorescens* strains (Fig. 1). A neighbour-joining dendogram was generated using the sequence from *Pseudomonas* sp. LPK2 and representative *Pseudomonas fluorescens* sequences from GenBank (Fig. 2). From this analysis, LPK2 aligned to same cluster (100% bootstrap value) with reference strain *Pseudomonas fluorescens* 2R37 (Fig. 2). This analysis has proven that LPK2 is strain of *Pseudomonas fluorescens* and can be designated as *Pseudomonas fluorescens* LPK2.

All the strains of *Pseudomonas* spp. (LPK1 to LPK8) were positive to IAA production with the development of pink colour with and without the addition of tryptophan into the culture media (Table 2). Tryptophan supplementation in the medium induced IAA production. This trend was common in all the strains of *Pseudomonas*. Most of the *Pseudomonas* strains produced HCN as evidenced by the change of the colour of filter paper from yellow to moderate and reddish brown (Table 2). *Pseudomonas* sp. LPK2 produced deep reddish colour as well as good activity of HCN, while LPK7 was negative for HCN activity (Table 2). In the presence of glycine and $FeCl_3$, the deep brown colour of filter paper was observed,

that was clear indication of induced HCN production by bacterial strains. All the strains of *Pseudomonas* spp. were screened for siderophore production on CAS agar. All the strains of *Pseudomonas* spp. produced siderophores forming orange halos around their spots on the CAS agar (Table 2). Maximum size of orange halo was produced by *P. fluorescens* LPK2. Secretion of siderophore by *P. fluorescens* LPK2 started after 12 h of incubation and lasted up to 5 days but on further incubation the production was declined (Fig. 2a). To know the type of siderophore, 48 h old supernatant of the strain LPK2 scanned at 400 nm showed major peak, corresponded with hydroxamate type of siderophores.

Chitinase activity was determined by the development of clear halo around the colonies or bacterial spots, while β -1,3-glucanase activity was confirmed by appearance of bacterial presence in the medium supplemented with laminarin as a sole source of carbon. *Pseudomonas* spp. LPK2, LPK4 and LPK8 showed chitinase activity (Table 2). *Pseudomonas* sp. LPK2 showed the maximum chitinase activity followed by LPK8 (Table 2). Chitinase formation was started after 24 h of incubation by *Pseudomonas* sp. LPK2 which reached maximum at 120 h and on further incubation its activity was declined (Fig. 2b).

In dual culture technique, all the strains of *Pseudomonas* (LPK1 to LPK8) inhibited the *in vitro* growth of *F. udum* (Table 2). Maximum inhibition of *F. udum* recorded in the case of *P. fluorescens* LPK2. It was recorded as 83% higher in comparison to control after 168h of incubation (Fig. 3). Cell free culture filtrate of *Pseudomonas* spp. LPK2 also restricted the hyphal growth of *F. udum* (Fig. 3). Morphological abnormalities in the hyphae of *Fusarium udum* obtained from the zone of interaction during dual cultures was observed by light microscopy and in scanning electron microscopy, revealed loss of structural integrity of the mycelium of *F. udum*. Twisting, hyphal perforations, lysis and empty cell formation, septation, fragmentation and degradation of mycelia of *F. udum* was observed under light microscopy (Fig. 4). Fragmentation and degradation of mycelia and loss of structural integrity of conidia of *F. udum* were clearly observed under Scanning electron microscopic analysis (Fig. 5). These features of mycelia may be due to production of lytic enzymes by strains LPK2.

Volatile compounds produced by *Pseudomonas* sp. LPK2 exhibited antagonistic activity when challenged against *F. udum* after 5 days of incubation. This strain caused 45% reduction in vegetative growth of *F. udum* (Fig. 6). On further incubation zone of inhibition of fungal pathogen decreased. Mycelial growth of both the pathogens decreased significantly in dual cultures by the antagonists with respect to control in iron deficient conditions (Fig. 7). In iron supplemented ($30 \mu M Fe^{3+}$) medium inhibition of the pathogenic fungi was reduced. In this case, *Pseudomonas* sp. LPK2 reduced fungal growth

maximally. However, percent inhibition was lowered in 60 μM Fe^{+3} supplemented medium in comparison to normal conditions of the medium. Under iron-deficient conditions remarkable inhibition of *F. udum* was recorded. It was found that under iron-deficient condition *Pseudomonas* sp. LPK2 inhibited 65% vegetative growth of *F. udum* (Fig. 7). On the other hand, the inhibition of the pathogen by *Pseudomonas* sp. LPK2 under iron-sufficient conditions was 51% (Fig. 7).

Discussion

Fusarium is a group of filamentous fungi widely distributed in various soil types. This is a large cosmopolitan genus of imperfect fungi and is of primary interest because numerous species are important plant pathogens (Nelson, 1991). *Fusarium* spp. occur frequently among the fungal microflora associated with seedling disease, and wilt is a major cause of plant mortality in many important crops (Minton and Garber, 1983). Isolates of *Fusarium* produce colony growth with fluffy, appressed or intermediate types of mycelium. Key macroconidial characters are shape, size and septation that distinguish the isolates from each other.

Due to wide distribution of different *Pseudomonas* spp. in the soil environment and relatively easy cultivation, genus *Pseudomonas* is among the best-studied bacterial group worldwide (reviewed by Kumar *et al.*, 2005). Some noble strains of *Pseudomonas aeruginosa* were isolated from our group from the rhizosphere of potato (Gupta *et al.*, 2002) and Sunflower (Bhatia *et al.*, 2005). Keeping this fact, more strains of fluorescent pseudomonads having PGP characters, were isolated from the rhizosphere of tomato (*Lycopersicon esculentum*).

A number of workers (Gupta *et al.*, 2001; Validov *et al.*, 2005) isolated, identified and characterized fluorescent pseudomonads from the rhizosphere of agricultural crops such as potato, tomato, cotton and tea. Initially, Kloepper *et al.* (1988) identified it as plant growth promoting rhizobacteria (PGPR) due to its rapid and aggressive rhizosphere colonization that proved as most powerful inoculants to improve plant growth and grain yield (Lucy *et al.*, 2004).

The morphological, physiological, and biochemical characteristics for the identification of rhizospheric pseudomonads (LPK1 to LPK8) followed the characteristics mentioned by Palleroni (1984) and Holt *et al.* (1994). The strains identified as fluorescent pseudomonads belong to *Pseudomonas fluorescens* and *P. aeruginosa* showed blue green fluorescent colour due to the presence of pyocynin pigment (Paulitz and Loper, 1991). The organism losses its pigment production under iron sufficient condition due to inhibition of siderophores production such as pyoverdine (Paulitz and Loper, 1991).

Five strains of *Pseudomonas* spp. (LPK1 and LPK5) were characterized according to 16S rDNA sequence

homology. All the strains were showing maximum sequence homology with their respective cluster of reference strains. In Phylogenetic tree based on 16S rDNA homology *Pseudomonas* sp. LPK2 is placed in *Pseudomonas fluorescens* clade. The use of 16S rDNA gene analysis has become promising. This is realized that 16S rDNA homology allows a first Phylogenetic affiliation of new isolate. Molecular microbiological characteristics have been carried out to characterize different groups of bacteria by using 16S rDNA sequencing.

The direct plant growth occurs due to Indole Acetic Acid (IAA) production (Chabot *et al.*, 1998), phosphate solubilisation (Nautiyal *et al.*, 2000), siderophores production (Gupta *et al.*, 2001) and HCN production (Antoun *et al.*, 1998), whereas indirect plant growth-promotion occurs due to growth restricting conditions by producing antagonistic substances such as antibiotics (Kang *et al.*, 2004), lytic enzymes (Lim and Kim, 1995). A bacterium can affect plant growth by one or more of these mechanisms and used different abilities for growth-promotion at various times during the life cycle of plants (Sessitsch *et al.*, 2005).

Interestingly, all the strains of *Pseudomonas* spp. produced siderophore which was agreement with the findings of Bhatia *et al.* (2005). The study further supported by Sindhu *et al.* (1999) who found 83% the fluorescent pseudomonads as siderophores producer while their quantities, in the form of orange halo vary. *P. fluorescens* LPK2 showed hydroxamate type of siderophores production. In case of LPK2 absorption maxima was recorded at 400 nm. Similar observations especially from fluorescent pseudomonads have been made by several workers (Gibson and Magrath, 1969). Iron in the soil is unavailable for direct assimilation by microbes because of its unavailable form i.e., ferric iron or Fe^{+3} , which is prominent in the soil and sparingly soluble about 10^{-18} M at pH 7.4 (Neilands and Nakamura, 1987). Siderophores secreted by PGPRs have been shown to have a very high affinity for iron and bind most of the Fe^{+3} that is available in the rhizosphere, and prevent the pathogens present in immediate vicinity from proliferation because of lack of iron (O' Sullivan and O' Gara, 1992).

Inhibitory effect of *P. fluorescens* LPK2 was observed against *F. udum* under iron-sufficient. It was found that inhibitory activity of strains of *P. fluorescens* LPK2 was partially neutralized when iron was supplemented to their respective medium. Significant reduction in the fungal inhibition has also been evidenced by Fuhrmann and Wollum (1989). Microbial siderophore production generally seems to be switched on when the Fe^{+3} concentrations is less than 20 μM (Lynch, 1990). In the present study, LPK2 examined for inhibition of *F. udum* under iron (30 μM Fe^{+3}) supplemented conditions showed reduction in inhibition against both the fungal pathogens. On increasing the concentration of iron to 60 μM Fe^{+3} , more inhibition was recorded in comparison to normal iron condition. Similar

findings were obtained by Derylo and Skorupska (1993) who reported that $60 \mu\text{M Fe}^{3+}$ suppressed the siderophore production in *Pseudomonas* sp. This gives evidence that one of the mechanisms for the inhibition of *F. udum* was due to the involvement of siderophores produced by *P. fluorescens* LPK2.

Harrison *et al.*, (1995) observed HCN production in *Pseudomonas aureofaciens* and one strain of *P. putida* from take-all suppressive soil. HCN is formed stoichiometrically in an oxidative reaction catalyzed by HCN synthase and its activity induced by glycine the precursor of HCN (Castric, 1981). Glycine amendment in the culture media of *P. fluorescens* LPK2 enhanced the quantity of HCN production showed its induced behaviour. HCN is a secondary metabolite produced by some Gram-negative bacteria such as *P. fluorescens* and *P. aeruginosa*, *Chromobacterium violaceum* (Askeland and Morrisom, 1983) and have a deleterious effect on growth of pathogens.

HCN and other volatile compounds produced by *P. fluorescens* LPK2 exhibited antagonistic activity on the growth of *F. udum* and caused growth inhibition of *F. udum*. The mode of action of HCN in disease control is partially understood but it is believed that cyanide ions derived from HCN are the potent inhibitor of many metallo-enzymes, especially copper-containing cytochrome C oxidase (Blumer and Haas, 2000) which are critical in respiration of living beings.

In our observations, *P. fluorescens* LPK2 released extracellular chitinase and β -1,3-glucanase enzymes. Chitinolysis plays an important role in biological control of plant diseases and has been substantiated with increased disease control by chitin-supplemented application of chitinolytic biocontrol agents. Chitinase and β -1, 3-glucanase are key enzymes in the decomposition of fungal hyphal wall (Rangel-Castro *et al.*, 2002). In the present study, It is thought that cell wall lysis occurred by concerted action of both chitinase and β -1,3-glucanase. chitinase since chitin and β -1,3-glucan are known to be the major components of most fungal cell walls (Lim and Kim, 1995). It has been also demonstrated that extracellular chitinase and laminarinase synthesized by *Pseudomonas stutzeri* digest and lyse mycelium of *F. solani* (Lim *et al.*, 1991).

Microscopic abnormalities in fungal pathogens under the influence of *P. fluorescens* LPK2 clearly showed degeneration and degradation of the hyphae of *F. udum*. Abnormal hyphal swelling, digestion of fungal cell wall, cytoplasm coagulation, hyphal perforation, hyphal tip degradation and halo cells formation appeared in mycelia of *F. udum* were caused by *P. fluorescens* LPK2 mediated by HCN and chitinolytic enzymes. Losses of structural integrity of conidia were also observed. Thus, it showed the hyphal wall interaction with bacterial cells rapidly lysed the hyphae, causing leakage of the fungal cytoplasm. Gupta *et al.* (2001) have been demonstrated the loss of sclerotial integrity, hyphal shriveling, mycelial and sclerotial

deformities and hyphal lysis in *M. phaseolina* by *Pseudomonas* GRC. Microscopic studies revealed the mode of the action of antagonistic activity and help to localize site of interaction between antagonists and fungal pathogens. *In vitro*, fungal growth inhibition assay suggested that antagonistic nature of *P. fluorescens* LPK2 against *F. udum* mainly due to production of antimicrobial substances, such as chitinolytic enzymes, HCN, antibiotics, siderophore and nutrient competition. However, role of other inhibitory metabolites such as toxins and proteolytic enzymes in the inhibition process of fungal pathogens can not be ruled out (Dunne *et al.*, 1998). *P. fluorescens* LPK2 showed inhibition of *F. udum* in cell free culture filtrates. There are several factors involved in the microbial antagonism which including staling growth products, pH of media, growth rate of interacting microbes, competition for nutrients and space, competitive colonization, nature and chemical composition of growth media, ionic or nutrient imbalance caused due to nutrient impoverishment and physical factors. Many researchers have attempted the development of biocontrol agents against soil-borne plant pathogens since the discovery of the PGPR by Kloepper *et al.* (1980).

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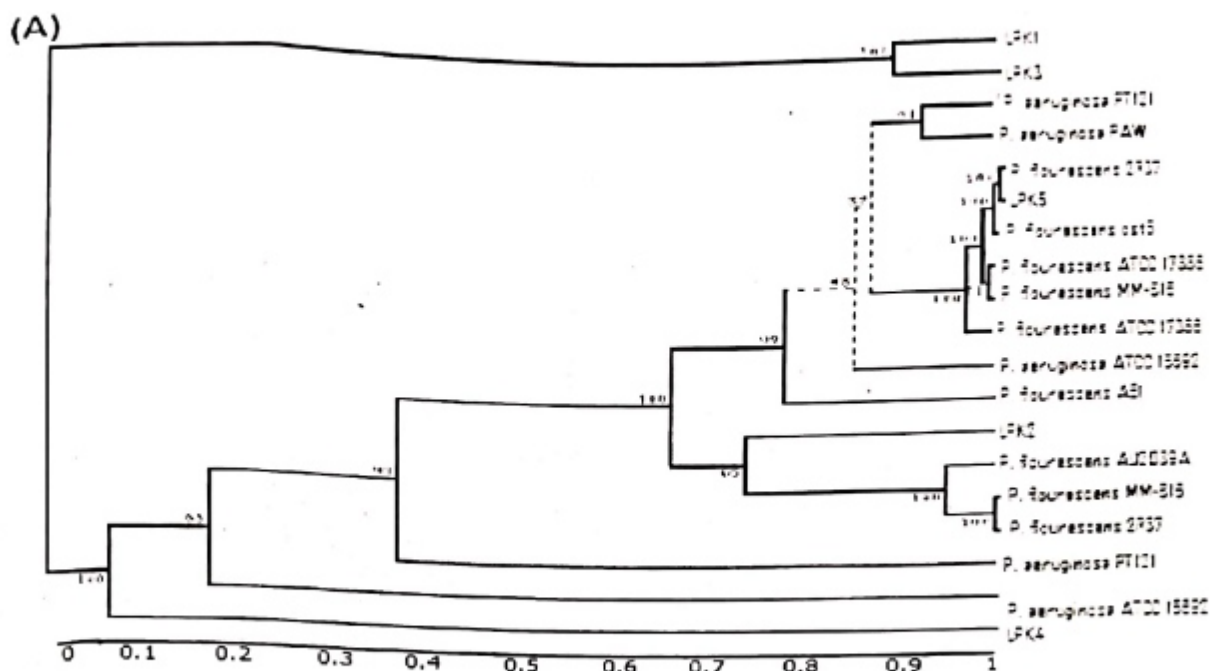
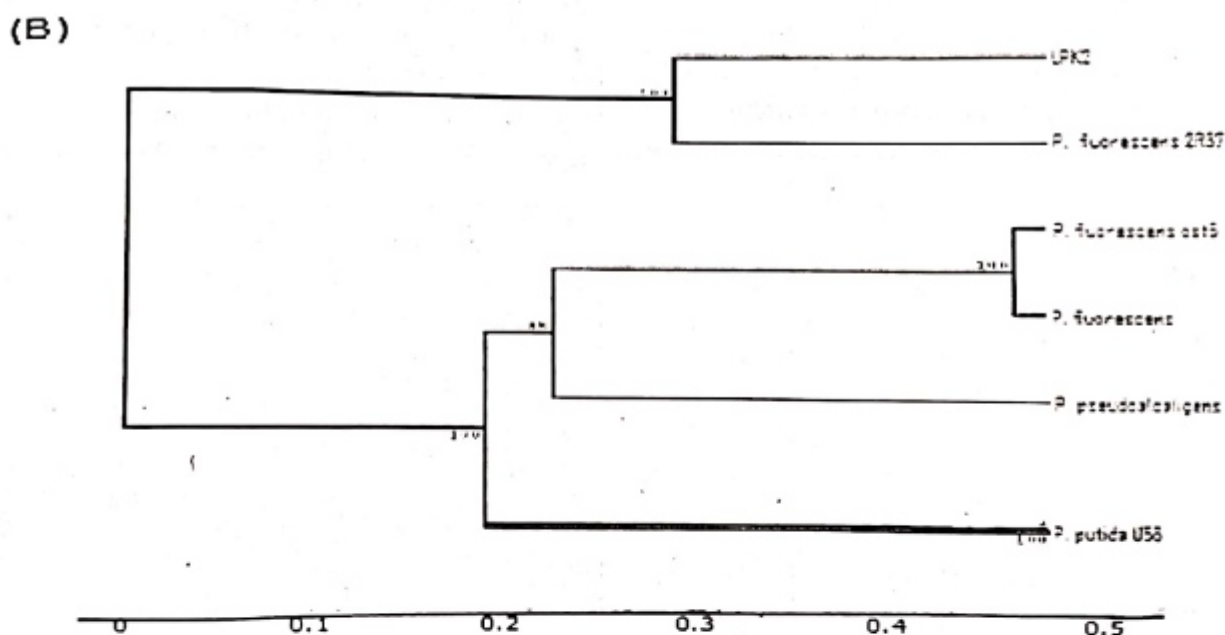


Fig 1: Time course analysis of siderophore production (A) and chitinase production (B) by *Pseudomonas* sp. LPK2 (Values are means of standard deviation of three replications)



Accession numbers represented: *Pseudomonas fluorescens* 2R37 (EF178447); *Pseudomonas fluorescens* ost5 (DQ439976); *Pseudomonas fluorescens* (Ab091837); *Pseudomonas pseudoalcaligenes* (AF181570); *Pseudomonas putida* U58 (Ef194102)

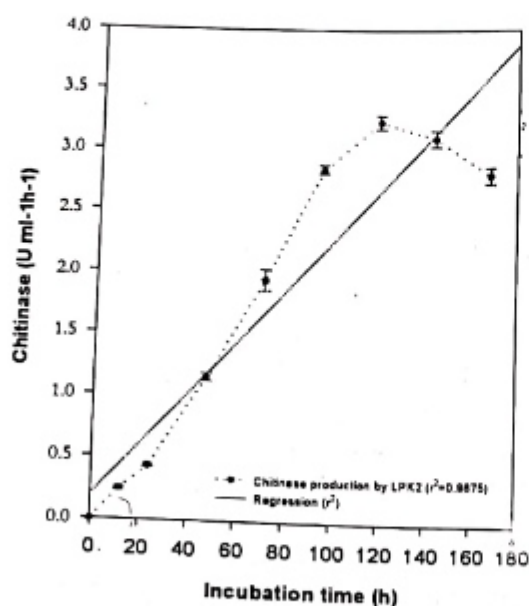
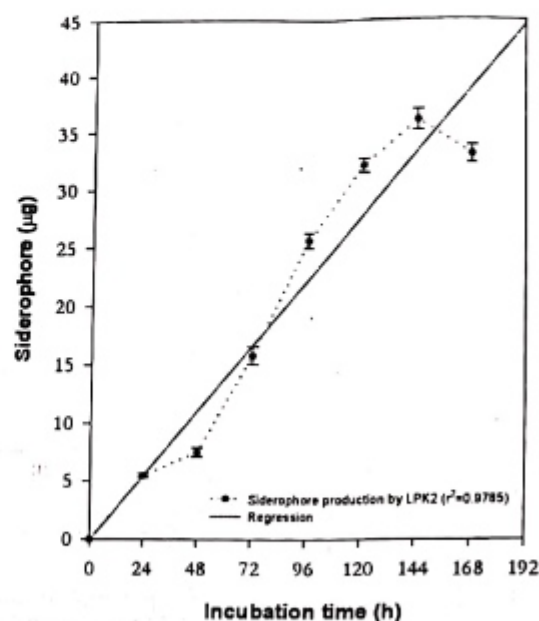


Fig 2: (A) Phylogenetic relationship between bacterial strains of *Pseudomonads* and representing species based on partial length 16S rDNA sequences constructed using cluster algorithm. Bootstrap values are indicated (from 1000 replication). Accession



(B) Phylogenetic relationship between *Pseudomonas* sp. LPK2 and representing species based on partial length 16S rDNA sequences constructed using cluster algorithm. Bootstrap values are indicated (from 1000 replication). Accession numbers are reported in text

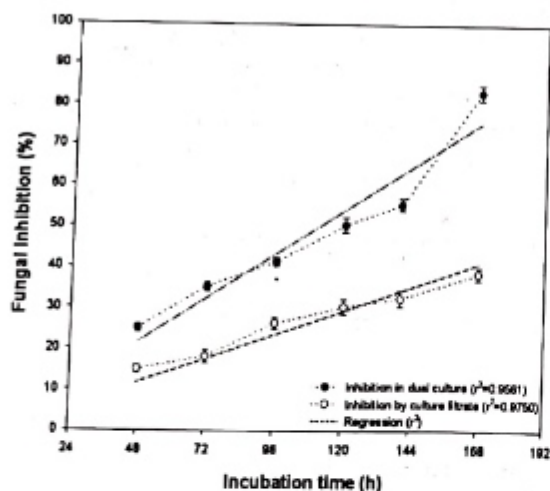


Fig 3: Inhibition of *F. udum* by *P. fluorescens* LPK2 (B) under dual culture and by cell free culture filtrate (Values are means of standard deviation of three replications)

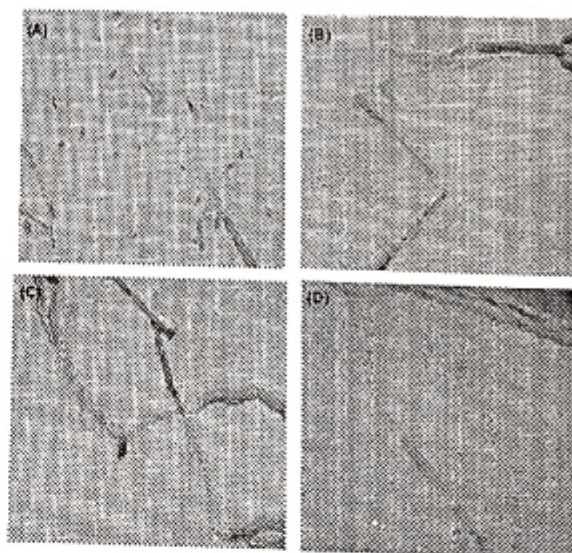


Fig 4: Compound microscopic photographs of post interaction events in *F. udum* due to *P. fluorescens* LPK2; halo cell formation and hyphal perforation (A), hyphal tip degradation (B), digestion of fungal cell wall (C) and halo cell formation (D) (see arrows)

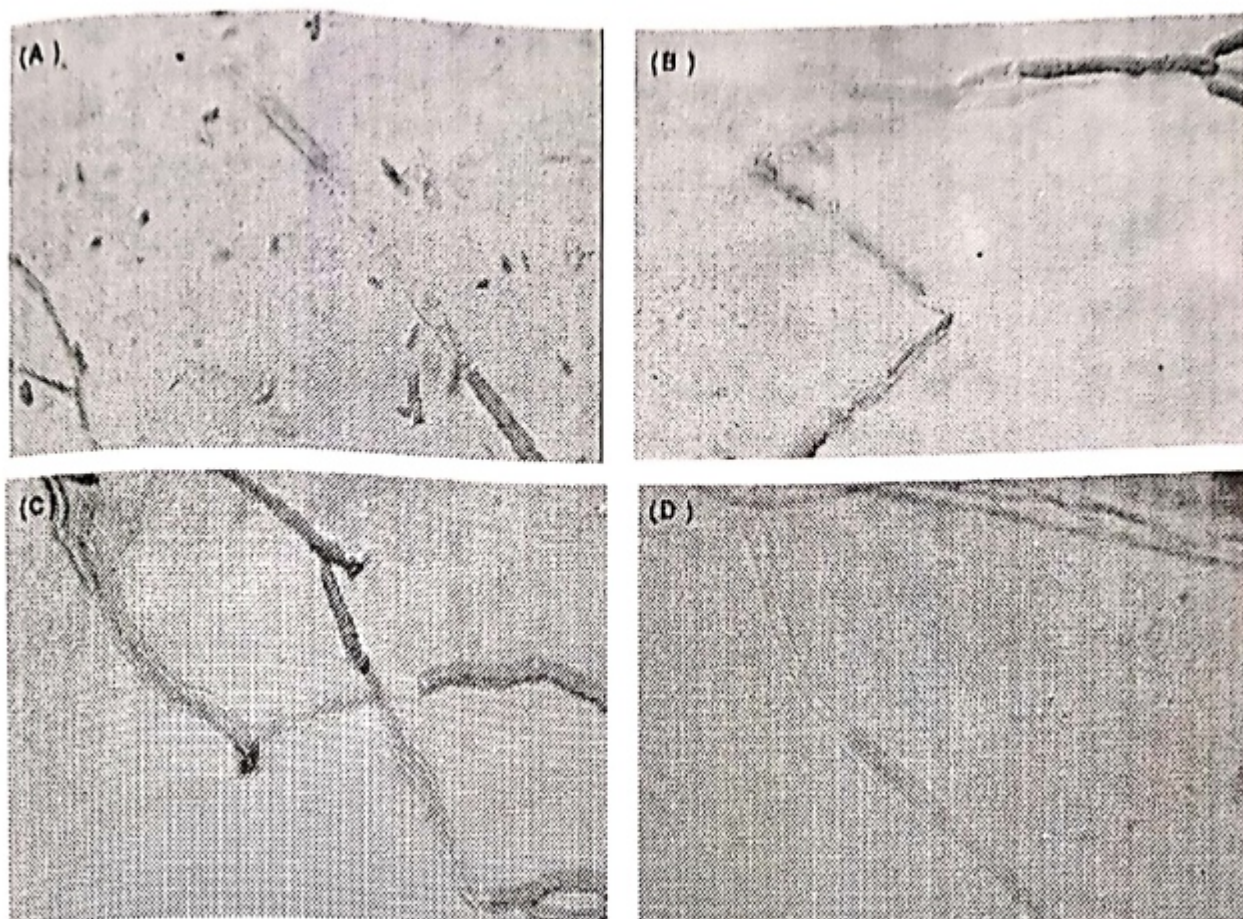


Fig 5: Scanning electron microscopic photographs of mycelial and conidial destruction by antagonistic interaction effects of *P. fluorescens* LPK2 control (A), hyphal lysis (B), hyphal destruction (C), hyphal and conidial lyses (D) (see arrows)

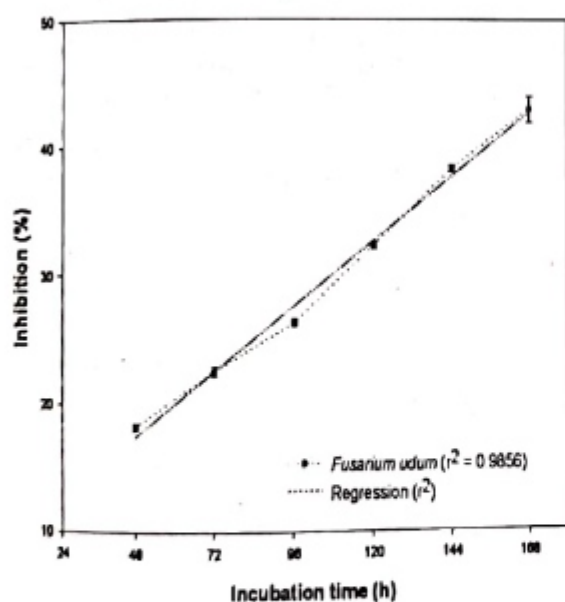


Fig 6: Effect of volatile compounds of *P. fluorescens* LPK2 on the growth inhibition of *F. udum* [Values are means of standard deviation of three replications]

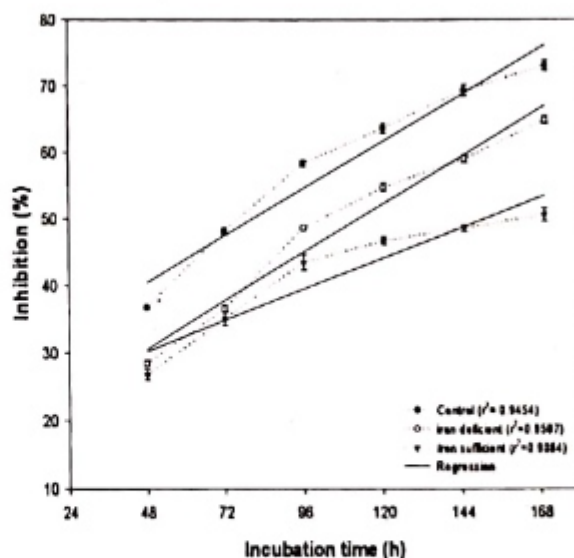


Fig 7: Antagonism between *F. udum* and *P. fluorescens* LPK2 under iron sufficient and iron deficient conditions (Values are means of standard deviation of three replications)

Table 1 Morphological, physiological and biochemical characters of fluorescent pseudomonads

Characteristics	LPK 1	LPK 2	LPK 3	LPK 4	LPK 5	LPK 6	LPK 7	LPK 8	1934	102	103
Gram reaction	-	-	-	-	-	-	-	-	-	-	-
Growth at: 4°C	-	-	-	-	-	+	-	-	+	+	+
41°C	+	+	+	+	+	+	+	+	+	+	+
Motility	+	+	+	+	+	+	+	+	+	+	+
Fluorescent diffusible pigment	+	(bg)	+	(bg)	+	(y)	+	(bg)	+	(bg)	+
Non-fluorescent non-diffusible pigment	-	-	-	-	-	-	-	-	-	-	-
Capsule	-	-	-	-	-	-	-	-	-	-	-
Endospore	-	-	-	-	-	-	-	-	-	-	-
PHB accumulation	-	-	-	-	-	-	-	-	-	-	-
Generation time (h)	1.4	1.3	1.3	1.4	1.4	1.3	1.4	1.2	1.2	1.3	1.3
Catalase	+	+	+	+	+	+	+	+	+	+	+
Oxidase	+	+	+	+	+	+	+	+	+	+	+
Urease	+	+	+	+	+	+	+	+	+	+	+
H ₂ S production	-	-	-	-	-	-	-	-	-	-	-
M. R. test	-	-	-	-	-	-	-	-	-	-	-
V. P. test	-	-	-	-	-	-	-	-	-	-	-
Gelatin hydrolysis	+	+	+	+	+	+	+	+	+	+	+
Starch hydrolysis	+	+	+	+	+	+	+	+	+	+	+
Arginine hydrolysis	+	+	+	+	+	+	+	+	+	+	+
Citrate utilization	+	+	+	+	+	+	+	+	+	+	+
Utilization of:											
Glucose	+	+	+	+	+	+	+	+	+	+	+
Meso-Inositol	-	-	-	-	-	-	-	-	-	-	-
Maltose	+	+	+	+	+	+	+	+	+	+	+
Mannitol	+	+	+	+	+	+	+	+	+	+	+
Mannose	-	-	-	-	-	-	-	-	-	-	-
Ribose	+	+	+	+	+	+	+	+	+	+	+
Trehalose	-	-	-	-	-	-	-	-	-	+	+

Abbreviations: +, positive; -, negative; PHB- poly hydroxyl butyrate; H₂S- hydrogen sulfide; MR- methyl red; VP-voges proskaur, bg- blue green; y- yellow; *Pseudomonas aeruginosa* MTCC-1934; *P. putida* MTCC-102 and *P. fluorescens* MTCC-103

Table 2 Plant growth-promoting and antifungal properties of *Pseudomonas* spp. strains

Strains	IAA ^A	Phosphate solubilization ^B	Siderophore ^C	HCN ^D	Chitinase ^E	β-1,3-glucanase	Antagonism against <i>F. udum</i>
LPK 1	+	+	+	+	+	+	+
LPK 2	++	++	+++	+++	+	+	++
LPK 3	+	+	+	-	-	+	-
LPK 4	+	+	+	-	+	-	-
LPK 5	+	++	+	++	-	+	-
LPK 6	-	+	+	-	-	-	-
LPK 7	++	++	++	-	++	+	+
LPK 8	-	+	+	+	-	+	+
Standard strains							
MTCC 1934	-	-	+	-	-	-	-
MTCC 102	-	+	-	-	-	+	-
MTCC 103	-	-	-	-	-	+	-

Abbreviations: A -, IAA negative, +, IAA positive; B -, Phosphate solubilization negative; +, phosphate solubilization positive; C -, Absence of halo formation; +, small halos <0.5 cm wide surrounding colonies; ++, medium halos > 0.5 cm wide surrounding colonies; +++, large halos >1.0cm wide surrounding colonies; D -, HCN negative, +, HCN positive; E -, chitinase negative, + chitinase positive; F -, β-1,3-glucanase negative, +, β-1,3-glucanase positive; *Pseudomonas aeruginosa* MTCC-1934; *P. fluorescens* MTCC-103; *P. putida* MTCC-102

Genetic variability, heritability and genetic advance in bottle gourd [*Lagenaria siceraria* (Mol.) standl.]

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Abstract

Extent of variability, heritability and genetic advance of fruit yield and its contributing characters were estimated in twelve diverse genotypes of bottle gourd. The study indicates that there is considerable amount of genetic variability for all the characters studied. The length of edible green fruit exhibited highest value of genotypic and phenotypic coefficient of variations (GCV and PCV). High estimates of heritability, genotypic coefficient of variations and genetic advance as per cent of mean were observed for length of edible green fruit, number of fruits plant⁻¹ and weight of edible green fruit. This indicates that these characters in bottle gourd can effectively be improved through selection.

Keywords : Bottle gourd, heritability, genetic advance.

Introduction

Bottle gourd (*Lagenaria siceraria* (Mol.) Standl.) is an important vegetable crop belonging to family cucurbitaceae and is commercially grown in almost all parts of India. It is a monoecious and highly cross-pollinated crop in which large amount of variations are observed in many economically important traits. The exploitation of genetic variability in the available genotypes is a prerequisite in a breeding programme for effective selection of superior genotypes. Since natural genetic variation for most of the yield attributes is considerably high in bottle gourd. There is an urgent need of information on the nature and magnitude of variation available in the material and part played by environment in expression of different characters. Keeping in view the above facts the present investigation was undertaken to estimate the magnitude of heritable and non-heritable components of variation and genetic parameters such as coefficient of variations, heritability and genetic advances in twelve diverse genotypes of bottle gourd.

Material and methods

Twelve diverse genotypes of bottle gourd having distinct diversity in morphology and performance were sown in randomized block design with three replication during rainy season at Vegetable Research Farm of the Department of Horticulture, Institute of Agricultural Sciences, Banaras

Hindu University, Varanasi. The spacing between row to row was kept at 2m and plant to plant was 1m in a 5m x 4m-plot size. The crop was maintained according to standard agronomic practices. Observations were recorded on five randomly selected plants for 14 different characters including vegetative as well as fruit characters such as days to germination, days to first staminate flower anthesis, days to first pistillate flower anthesis, number of primary branches plant⁻¹, vine length, node number bearing first staminate flower, node number bearing first pistillate flower, days to first fruit harvest, length of edible green fruit, number of fruits plant⁻¹, length of edible green fruit, number of fruits plant⁻¹, weight of edible green fruit, number of seeds fruit⁻¹, 100-seed weight and edible fruits yield plant⁻¹. The statistical analysis was carried out as per formula suggested by Panse and Sukhatme (6) and was used for calculating other genetic parameters. Genotypic and phenotypic coefficients of variations were calculated as per the formula suggested by Burton and Devane (2). Heritability in board sense and genetic advance were calculated as per the formula given by Allard (1) and Johnson *et.al.* (3), respectively.

Results and discussion

The extent of variability present in twelve diverse genotypes of bottle gourd was measured in terms of range, mean, variations (PCV and GCV), heritability (broad sense) and genetic advance (GA) and GA as per cent of mean [Table-1]. All the genotypes differed significantly with respect to different characters studied. Wide range of

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variation was observed in all the characters studied. Number of fruits plant⁻¹ varied from 3.83 (VRBG-40) to 7.20 (VRBG-101), length of edible green fruits from 28.97cm (PGOG-62) to 60.00cm (VRBG-110) and fruits yield plant⁻¹ from 5.33kg (PBOG-40) to 9.63kg (VRBG-110) (Table-2). Prakash *et al.* (7) also reported wide range of variation for most of the characters studied in this crop.

The GCV, which gives a picture of extent of genetic variability in the population ranged from 5.21 (days to first fruit harvest) to 23.68 (length of edible green fruit). The GCV values were considerably high for characters such as number of fruits plant⁻¹, weight of edible green fruit and fruits yield plant⁻¹. The characters having higher range of variation have a better scope for improvement through selection. This finding is in agreement with those reported by Tyagi (4). A perusal of data in table-1 shows that there was close relationship between PCV and GCV and was observed low for all the characters except vine length and fruits yield plant⁻¹ which indicated less influence of environment for most of the characters.

With the help of GCV alone, it is not possible to determine the amount of variation that is heritable. Heritable variation can be found out with greater degree of accuracy. The heritability estimates of 100-seed weight (98.90 %) was highest and was moderate for vine length (71.50%) and number of primary branches plant⁻¹ (71.00%) which suggest that the environmental effects constitute a major portion of total phenotypic variation and hence direct selection for this characters will be less effective. Expected genetic advance and its estimates as per cent of mean for various characters revealed that length of edible green fruit (48.21), weight of edible green fruit (43.60) and number of fruits plant⁻¹ had high GA. High heritability along with high expected GA were recorded for length of edible green fruit

and 100-seed weight which was also recorded by findings of Prasad and Prasad (5). If heritability was mainly due to non-additive gene action, the expected GA could be expected. It is clear from table-1 that the characters like length of edible green fruit and number of seeds fruit⁻¹ possessing high heritability along with GA could be effectively used for selection as it is also suggested by Johnson *et al.* (3). High heritability along with low GA was observed in characters namely number of seeds fruit⁻¹, days to first pistillate flower anthesis and days to first fruits harvest indicated that expression of these characters was governed by non-additive gene action.

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Table 1 Range, Mean, Variability, Heritability and Genetic Advance for 14 characters in bottle gourd

Table 1 Range, Mean, Variability, Heritability and Genetic Advance for 14 characters in bottle gourd									
S. No.	Characters	Range	Mean	Variability		Heritability (%) (Broad Sense)	Genetic Advance (GA)	GA as per cent of mean	
				*GCV	**PCV				
1	Days to germination (50%)	7.43-11.00	9.17	11.87	12.35	89.80	2.13	23.22	
2	Days to first male flower anthesis	42.97-53.83	47.82	7.24	7.34	97.50	7.05	14.74	
3	Days to first female flower anthesis	51.10-61.77	54.81	6.22	6.31	97.20	6.92	12.62	
4	No. of primary branches plant ⁻¹	3.83-6.30	4.85	14.28	16.96	71.00	1.20	24.74	
5	Vine length (m)	3.47-6.27	4.81	16.42	19.42	71.50	1.38	28.69	
6	Node no. to first male flower	5.50-7.97	6.86	9.91	12.03	67.90	1.15	16.76	
7	Node no. to first female flower	8.77-11.83	9.82	10.45	11.04	89.60	2.00	20.36	
8	Days to first fruit harvest	65.20-76.77	69.14	5.21	5.31	96.00	7.26	10.50	
9	Average length of edible green fruit (cm)	28.97-60.00	47.27	23.68	23.96	97.70	22.79	48.21	
10	Average no. of fruits plant ⁻¹	3.87-7.20	5.52	20.56	21.95	87.70	2.19	39.67	
11	Average weight of edible green fruit(kg)	0.87-1.87	1.33	22.34	23.87	87.60	0.58	43.60	
12	Average no. of seeds fruit ⁻¹	310-497.67	423.08	11.59	12.24	89.70	25.64	6.06	
13	100 seed weight(g)	11.57-15.33	13.69	8.22	8.27	98.90	2.31	16.87	
14	Average fruit yield plant ⁻¹ (kg)	5.33-9.63	7.03	18.15	23.36	60.40	2.04	29.01	

**PCV=Phenotypic coefficient of variability *GCV=Genotypic coefficient of variability

Table 2 Mean value of the twelve genotypes for fourteen characters in bottle gourd.

S. No.	Genotypes	Days to germination (50%)	Days to first male flower anthesis	Days to first female flower anthesis	No. of primary branches plant ⁻¹	Vine length (m)	Node no. to first male flower	Node no. to first female flower	Days to first fruit harvest	Average length of edible green fruit (cm)	Average no. of fruits plant ⁻¹	Average weight of edible green fruit (kg)	Average no. of seeds fruit ⁻¹	100 seed weight (g)	Average fruit yield plant ⁻¹ (kg)
1	VRBG-101	9.47	45.10	52.13	4.70	4.83	7.07	8.97	66.33	47.33	7.2	1.30	462.33	12.80	7.70
2	PBOG-40	8.80	47.67	54.57	5.37	3.47	7.43	10.73	70.33	43.93	4.83	1.10	400.00	12.57	5.33
3	NDBG-58	7.43	48.70	54.93	3.83	5.13	5.50	9.07	69.30	55.78	4.87	1.17	443.67	13.30	5.60
4	DVBG-1	8.63	44.83	51.83	4.77	5.53	6.53	9.90	65.20	57.97	4.83	1.43	479.00	14.60	6.87
5	PGOG-62	9.13	50.03	57.13	6.30	4.60	6.57	8.93	70.73	28.97	7.10	0.87	310.00	13.97	5.97
6	DVBG-2	8.87	45.03	51.67	3.93	4.07	7.97	10.83	65.73	30.37	6.07	1.00	396.67	14.13	6.07
7	PBOG-61	7.50	45.80	51.10	4.93	5.53	7.53	10.07	65.23	30.80	6.13	1.23	413.33	15.23	7.73
8	VRBG-110	8.87	50.87	57.03	5.03	4.00	5.97	8.93	66.53	60.00	6.83	1.43	497.67	12.87	9.63
9	PSPL	10.90	53.83	61.77	5.00	5.37	7.67	11.83	76.77	54.50	3.87	1.73	374.67	11.57	6.63
10	NDBG-56	11.00	53.07	58.63	4.80	6.27	6.77	8.77	73.80	52.20	5.10	1.87	429.33	14.33	9.60
11	VRBG-40	9.80	45.77	52.37	3.90	3.80	7.03	10.93	68.80	51.87	3.83	1.70	433.00	13.63	6.50
12	Pusa Naveen	9.73	42.97	53.63	5.73	5.17	6.30	8.90	71.00	53.67	5.63	1.23	457.33	15.33	6.77

Variability, heritability and character association analysis in garden pea (*Pisum sativum* L.)

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Abstract

Genetic variability and character association were studied for yield and its component characters in 20 genotypes of pea (*Pisum sativum* L.) during *rabi*, 2009-10. Analysis of variance revealed significant difference for all the characters indicating the presence of good amount of variability in the genotypes studied. The estimates of GCV, heritability and genetic gain were higher for plant height, whereas, high heritability with moderate GCV and genetic gain were observed for days to first flowering, days to 50 % flowering, days to first fruit setting, days to first pod picking and number of pickings, indicating good scope for selection. Association studies revealed that green pod yield per plant was positively and significantly associated with pod length, pod weight and number of grains per pod. The result suggested that these traits could be considered as major yield contributing traits in garden pea.

Key words: Garden pea, genetic variability, heritability, genetic advance, character association.

Introduction

Pea (*Pisum sativum* L.) is an important crop grown throughout the world. In India, it is grown mainly as winter vegetable in the plains of North India and as summer vegetable in the hills. Pea is used as fresh vegetable, canned, processed or dehydrated and seeds are consumed as pulse. Green pods are highly nutritive, containing high percentage of digestible protein (7.2%), carbohydrates (19.8%) and minerals (0.8%) (Aykroyd, 1963). Genetic variability plays an important role in selecting best genotypes for making rapid improvement in yield and other desirable characters. Heritability is an index for calculating the relative influence of environment on expression of genotypes. The expected genetic advance is important to have an idea of effectiveness of selection. Estimates of heritability together with genetic advance predict the expected progress to be achieved through selection. Correlation coefficient is a statistical measure, which is used to find out the degree and direction of relationship between two or more variables, it also measures the mutual relationship between various plant characters and determines the component characters on which selection can be exercised for genetic improvement in yield. Keeping in view the study was conducted to find out the extent of variability, heritability, genetic advance and character association for yield and its contributing traits in garden pea.

Materials and methods

The present investigation was carried out at Experimental farm, Department of Horticulture, S.K.N. College of Agriculture, Jobner, Rajasthan during *rabi* 2009-10. The experimental material consisted of twenty diverse pea genotypes namely, VRP-5, VRP-6, VRP-7, VRP-22, AP-1, VRP-343, VRP-90, VRP-216, VRP-158, VRP-238, VRP-147, VRP-16, AP-3, Arkel, VRP-179, VRP-135, VRP-219, VRP-330, VRP-138 and EC-9126. Experiment was laid out in randomized block design with three replications. The pea seeds were sown at a spacing of 30 cm x 10 cm. Recommended agronomic practices and plant protection measures were followed to maintain optimum plant stand. The observations were recorded on five randomly selected competitive plants from each plot in every replication for plant height (cm), days to first flowering, days to 50 % flowering, days to first fruit setting, days to first pod picking, number of pods per plant, pod length (cm), pod weight (g), number of grains per pod, number of pickings, pod yield per plant (g), pod yield per hectare (q), TSS of green seeds (%) and protein content in pods (%) (A.O.A.C., 1960). The data were averaged and statistically analyzed for analysis of variance as per the method suggested by Panse and Sukhatme (1995). The genotypic and phenotypic coefficients of variation were worked out as per the method suggested by Burton and De Vane (1953). The heritability in broad sense and expected genetic advance were worked out using formulae of Hanson *et al.* (1956) and Johnson *et al.* (1955). The genotypic and phenotypic correlation coefficients were calculated from the

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genotypic and phenotypic covariances and variances as described by Singh and Choudhary (1977) and as per formula given by Johnson *et al.* (1955).

Result and discussion

The analysis of variance (Table 1) indicated significant differences among the genotypes for all the observed characters which indicated that high amount of genetic variability was present in the genetic material. The extent of genotypic variability indicated the amenability of a given character for its improvement (Burton, 1952). Estimates of phenotypic coefficient of variation (PCV), genotypic coefficient of variation (GCV), heritability (h^2) in broad sense and genetic advance as percentage of mean for various characters are presented in table 1. The phenotypic coefficients of variation (PCV) were higher than the respective genotypic coefficients of variation (GCV) for all the characters which indicated the positive effect of environment in enhancing differences among the genotypes at phenotypic level. Phenotypic coefficient of variation (PCV) was highest for plant height (31.87%) followed by days to 50 per cent flowering (24.13%), days to first flowering (21.32%), days to first fruit setting (21.24%), pod weight (16.72%), number of pods per plant (16.42%) and days to first pod picking (15.25%) whereas, minimum value of PCV was recorded for protein content in pods (6.29%). The genotypic coefficient of variation (GCV) for plant height was maximum (30.18%), followed by days to 50 per cent flowering (23.88%), days to first flowering (20.97%), days to first fruit setting (20.94%) and days to first pod picking (15.05%) while, it was lowest for protein content in pods (4.30%). Similar results were earlier reported by Sureja and Sharma (2000) and Sardana *et al.* (2007).

High heritability (>75%) along with high genetic advance (>45%) was observed for plant height (89.65, 57.92%) and days to 50% flowering (97.94, 47.90%). Whereas, high heritability coupled with moderate genetic advance was recorded for days to first fruit setting (97.23, 41.86%), days to first flowering (96.74, 41.80%), days to first pod picking (97.40, 30.08%) and number of pickings (93.47, 28.04%). Low heritability and low genetic advance were recorded for number of grains per pod (46.23, 9.95%) and protein content (46.66, 5.97%) in the pod (Table 1). High heritability and moderate to high genetic advance for plant height, days to 50 per cent flowering, days to first fruit setting, days to first pod picking, days to first flowering and number of pickings indicated that effect of environment in expression of these traits was negligible as these traits were governed by additive gene action. Thus, these traits respond to selection owing to their high genetic variability and transmissibility. Low heritability coupled with low genetic advance was recorded for number of grains per pod and protein content in the pod indicated that expression of these traits significantly influenced by environment because, these traits were governed by non-additive gene action. These results were in close conformity with the earlier findings of Gupta *et al.* (2006), Sardana *et al.* (2007) and Sharma *et al.* (2007).

The correlation studies revealed that in general

estimates of genotypic correlation coefficients were higher than the corresponding phenotypic correlation coefficients, which indicated a strong inherent association among different traits under study (Table 2). The lower phenotypic values might be due to environmental interactions. A significant positive association of pod yield per plant was observed with pod weight (0.469), pod length (0.395) and number of grains per pod (0.392) at phenotypic level and with number of grains per pod (0.763), pod length (0.663), pod weight (0.542) and number of pickings (0.376) at genotypic level, which, suggested that these characters could be considered as major green pod yield contributing characters in pea. Similar results were earlier obtained by Chaudhary and Sharma (2003), Sureja and Sharma (2004), Choudhary *et al.* (2004) and Nawab *et al.* (2008). Pod yield per plant registered significant and negative correlation with days to first flowering (-0.326) and days to first pod picking (-0.369), which, indicated that selection should be practiced for less days to flowering and first fruit setting (earliness). The results were in accordance with Chaudhary and Sharma (2003), Choudhary *et al.* (2004) and Sureja and Sharma (2004).

The present investigation revealed that wide variability existed for different morphological traits in the germplasm of pea. High heritability coupled with high genetic advance was noticed through plant height and days to 50% flowerings, suggesting that these characters could be considered reliable indices for selection, to enhance the pod yield. Correlation studies revealed that pod yield was significantly and positively associated with pod weight, pod length and number of grains per pod. Similarly a significant and negative association of pod yield was observed with days to first fruit setting and days to first flowering, which suggested that for these traits will help for improvement in pod yield in garden pea. The negative association for these two traits with pod yield suggested that selection for earliness should be practiced to improve the yield.

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Table 1. ANOVA. Main range, genotypic and phenotypic coefficient of variation, heritability (hs) and genetic advance as percentage of mean for different characters in pea

Character	Replication		Genotypes		Error	d	Mean	Range	S.E.	Variance		Coefficient of variation		Heritability in broad sense (%)	GA	GAS % age d mean
	d		d							Genotypic	Phenotypic	Genotypic	Phenotypic			
PH	46.258		2356789		88.413		91.71	60.47-182.67	5.43	766.11	854.53	30.18	31.87	89.66	53.11	57.92
DF	3.016		24061		2.655		42.47	32.33-62.00	0.94	79.31	81.98	20.97	21.32	96.74	17.74	41.80
50%F	5.716		424255		2.944		49.62	35.33-69.33	0.99	140.43	143.38	23.88	24.13	97.94	23.76	47.90
DFFS	5.0		381705		3.578		53.60	40.67-71.67	1.09	126.04	129.62	20.94	21.24	97.23	22.43	41.86
DFFP	0.516		319062		2.814		68.22	53.67-84.00	0.97	105.41	108.23	15.05	15.25	97.40	20.51	30.08
PP	2.616		7.677		1.092		11.04	8.99-16.07	0.60	2.19	3.28	13.42	16.42	66.76	2.48	22.52
PL	0.0096		1.166		0.202		8.47	7.13-9.27	0.26	0.30	0.50	6.52	8.40	60.14	0.66	10.25
PW	0.253		1.541		0.198		4.81	3.50-6.17	0.26	0.44	0.64	13.91	16.72	69.26	1.13	23.64
GP	0.151		1.197		0.334		7.46	6.23-8.40	0.33	0.26	0.62	7.19	10.57	46.23	0.74	9.95
Pickings	0.0166		0.732		0.0166		3.42	3.00-4.00	0.07	0.23	0.25	14.29	14.78	93.47	0.95	28.04
YP	12.712		48.998		10.307		45.96	40.41-52.87	1.85	13.22	23.53	7.91	10.55	56.20	5.57	12.13
Yha	52.777		231.855		44.046		96.06	79.21-110.12	3.83	62.61	106.66	8.32	10.86	58.70	12.36	13.03
TSS	1.023		19.0255		2.430		23.39	19.63-28.50	0.90	5.53	7.96	10.05	12.06	69.48	3.97	17.02
Protein	0.0281		0.466		0.136		8.05	7.26-8.86	0.21	0.12	0.25	4.30	6.29	46.66	0.48	5.97

PH= Plant height (cm), DFF= Days taken to first flowering, 50%F= Days to 50% flowering, DFFS= Days to first fruit setting, DFFP= Days to first pod picking, PIP= Number of pods per plant, PL= Pod length (cm), PW= Pod weight (g), GP= Number of grains/pod, Pickings= Number of pickings, YP= Yield/plant (g), Yha= Yield/ha (g), TSS= T.S.S of green seeds (%), Protein= Protein content in pods (%)

Table 2. Phenotypic correlation coefficients (upper diagonal) and genotypic correlation coefficients (lower diagonal) between different traits in pea

S. No.	Traits	PH	DFF	50% F	DFFS	DFPP	P/P	PL	PW	G/P	Picking	TSS	Protein	Y/P	Y/ha
1	PH	1.000	0.559**	0.657**	0.631**	0.712**	0.739**	-0.477**	-0.649**	-0.265	-0.602**	0.435**	-0.136	-0.096	-0.039
2	DFF	0.597	1.000	0.933**	0.896**	0.944**	0.274	-0.200	-0.483**	-0.0811	-0.699**	0.3643*	0.221	-0.326*	-0.251
3	50% F	0.694	0.935	1.000	0.961**	0.953**	0.437**	-0.299	-0.594**	-0.142	-0.614**	0.324*	0.166	-0.318	-0.243
4	DFFS	0.686	0.912	0.977	1.000	0.936**	0.471**	-0.349*	-0.649**	-0.220	-0.665**	0.437**	0.136	-0.369*	-0.292
5	DFPP	0.751	0.949	0.955	0.951	1.000	0.459**	-0.304	-0.595**	-0.1159	-0.759**	0.438**	0.130	-0.271	-0.182
6	P/P	0.933	0.364	0.561	0.573	0.5782	1.000	0.517**	-0.785**	-0.345*	-0.380*	0.342*	-0.182	0.070	0.106
7	PL	-0.666	-0.293	-0.415	-0.499	-0.421	-0.779	1.000	0.602**	0.664**	0.318	-0.259	0.342*	0.395*	0.366*
8	PW	-0.828	-0.601	-0.739	-0.791	-0.734	-0.835	0.966	1.000	0.488**	0.4758**	-0.368*	0.177	0.469**	0.438**
9	G/P	-0.475	-0.140	-0.236	-0.351	-0.195	-0.527	0.815	0.776	1.000	0.225	-0.306	0.182	0.393*	0.400*
10	Picking	-0.664	-0.735	-0.643	-0.693	-0.792	-0.421	0.363	0.587	0.285	1.000	-0.632**	0.102	0.246	0.164
11	TSS	0.583	0.454	0.397	0.528	0.543	0.506	-0.325	-0.576	-0.419	0.745	1.000	-0.013	-0.151	-0.100
12	Protein	-0.255	0.308	0.216	0.194	0.172	-0.163	0.517	0.168	0.231	-0.092	-0.065	1.000	0.234	0.246
13	Y/P	-0.211	-0.488	-0.440	-0.511	-0.378	-0.017	0.664	0.542	0.764	0.377	-0.271	0.296	1.000	0.986**
14	Y/ha	-0.125	-0.329	-0.331	-0.398	-0.252	0.043	0.599	0.485	0.760	0.257	-0.188	0.310	0.977	1.000

PH = Plant height (cm). DFF = Days taken to first flowering. 50% F = Days to 50% flowering. DFSS = Days to first fruit setting. DFPP = Days to first pod picking. P/P = Number of pods/plant. PL = Pod length (cm). PW = Pod weight (g). G/P = Number of grains/pod. Pickings = Number of pickings. Y/P = Yield/plant (g). Y/ha = Yield/hectare (q). TSS = T.S.S. of green seeds (%). Protein = Protein content in pods (%).

- ** Significant at P = 0.01 level of significance
- * Significant at P = 0.05 level of significance

Seasonal variation in characteristics of underground irrigation waters and their influence on soils

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Abstract

Two sets of well water were collected, one before the monsoon rains in the month of April-May and second after the cessation of monsoon rains in the month of October. After the rains, salt concentration in the well water was reduced. Soil samples were also collected from areas irrigated by water of some of these wells, and analysed to study the effect of continuous use of irrigation water on them. Amount of salt retained in soil after the rains was linearly related to initial salinity in the soil. Being low rainfall in the studied area, the negative values of salt regime constant 'd' were also of very low order indicating very less leaching of salts from the crop root zone and thus, continuous use of higher salinity water might hinder the crop production.

Key words: seasonal variation, underground irrigation waters, influence on soils.

Introduction

The concentration and nature of the salts present in irrigation water determine its quality. The nature of salts in irrigation water, sometimes may be more important than the total amount of salts e.g. if the proportion of sodium in irrigation water is high, the soils may become gradually unproductive. Even water of doubt full quality can be used without serious damage to soils and crops, if the necessary precautions are taken in irrigation and soil management. The limits for maximum permissible salt content of water depend also on the nature of the salts and amount of rainfall. Saline irrigation water is better suited on soils which are permeable and where the water table remains 1.8 to 3 meters below the surface.

A large area in Bikaner district of Rajasthan is irrigated by tube wells. A critical study of the variation in quality of well waters and their influence on soils in long use was under taken.

Materials and methods

Water samples were collected from fifty six tube wells from twenty villages of Bikaner district of Rajasthan. The first set of samples was drawn before the monsoon in April – May 2004 and the second set of after the cessation of monsoon rains in October 2004. For salt balance studies soil samples (0-30 cm depth) from twenty selected sites

were also collected in month of April-May and October 2004 and salt balance in irrigated soils was calculated by equation given by Szabolcs (1969) as $d = b - [a + (cv/mds) \times 10^{-5}]$ where,

d = salt leached or accumulated, g/100 g soil

b = amount of salt at end of observation, g/100 g soil

a = amount of salt at start of observation, g/100 g soil

c = salt content in rain water, g/L

v = amount of monsoon rains, mm

m = thickness of soil layer, m

ds = bulk density of soil, mg/m³

Water and soil samples were analysed using standard methods and procedures. Soils of the studied area are aeolian, coarse textured, calcareous and alkaline in nature with low fertility status, low water holding capacity and susceptible to wind erosion

Results and discussion

The analytical data of tube well waters are presented in table 1. Value under A relate to the pre-monsoon period and values under belated post- monsoon period. Calculated valued of SAR, RSC and pH, EC are given in table 2. Soil data are presented in table 3 and 4.

Data presented in table 1 indicate that the tube well waters contain relatively high sodium, chloride and bicarbonate barring a few exceptions. Another feature about these waters is that magnesium is generally higher than calcium. After the monsoon rains, salt concentration in the tube well waters were reduced. The ions which have decreased are sodium, Chloride and sulphate while,

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magnesium, bicarbonate are increased and calcium and carbonate ions not showed much difference. P^H values of all irrigation waters were noticed to fluctuate during April-May to October (Table-2). The mean P^H value of water samples was increased from 8.30 to 8.44 during April-May to October. The increase in P^H of these irrigation waters during rainy season might be due to increase in concentration of neutral salts of calcium and magnesium. The results get support from findings of Gupta *et al.* (1982).

Electrical conductivity of underground irrigation waters of Bikaner district showed decreasing trend from April-May to October (Table 2). The mean value of EC of irrigation waters was decreased from 2.58 to 2.02 dSm⁻¹. Systematic decreasing trends was observed in EC of waters between the periods of April-May to October. The highest and lowest EC values of all the samples were observed during the months of April-May and October, respectively. The EC of irrigation waters was decreased 21.71 per cent during the observation period. Owing to the rain water induced dilution and leaching of salts the electrical conductivity decreased during the rainy season. Similar results were also reported by Kameariya (1977).

It is evident from the data presented in table 2 that a systematic decreasing trend in SAR values was observed in all irrigation waters between the period of April-May to October. The highest SAR values of all waters were observed during the month of April-May, while the lowest values were observed during the month of October. The mean SAR value of irrigation waters of Bikaner district was decreased from 14.84 to 9.77 (34.16 per cent) during the study period. The decrease in SAR value during rainy season might be due to a decrease in the salt concentration. This showed that there was improvement in the quality of underground irrigation waters both in salinity and sodium hazards after monsoon rains. Similar finding were also reported by Gupta *et al.* (1982).

Residual sodium carbonate contents of all irrigation waters were observed minimum during the month of October and maximum during the months of April-May (Table 2). The decrease in mean RSC values of irrigation waters of Bikaner district was recorded from 1.30 to 0.44 me L⁻¹ during April-May to October. This showed that there was improvement in the quality of underground irrigation waters with respect to alkali hazards. This might be due to leaching of soluble salts and increase in neutral salts of calcium and magnesium during raining season. The results of present investigation get support from the findings of Datta and Taygi (1996).

Salt balance in plant root zone is very important. Water evaporates in a pure state leaving salts behind. As water is removed from the soil by evaporation/evapotranspiration the salt concentration in the soil solution is increased. Each irrigation adds some salts to soil depending upon the water entering the soil and the salt concentration in the water. This salts remains in the soil and accumulates unless it is leached away by rain water or water applied in excess of crop requirement. In the present study, salt removal by rain water was estimated using the equation given by Szabolcs (1969). Results of salts balance studies of soils of Bikaner district given in table 4 and discussed as follows. The negative values of salt regime constant 'd' (Table 4) indicated that the salt added through irrigation water was either leached down to lower layers of the soil profile or washed through the surface run off. It is further clean from the data that amount of salt retained in soil after the rains was linearly related to initial salinity in the soil. In other words, higher was the amount of accumulated salt in soil after the irrigation cycle, greater was the amount of salt leached/washed. However, being low rainfall in the studied area, the negative values of salt regime constant 'd' were also of very low order indicating very less leaching of salts from the crop root zone and thus continuous use of higher salinity water might hinder the crop production.

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Table 1. Chemical composition of tube well waters before and after monsoon (expressed as me L⁻¹) of Bikaner district

Characters	CO ₃ ⁺⁺		HCO ₃ ⁻		Cl ⁻		SO ₄ ⁻²		Ca ⁺⁺		Mg ⁺⁺		Na ⁺	
	A *	B **	A	B	A	B	A	B	A	B	A	B	A	B
Range	0.5-2.0	Nil-2.0	0.75-5.5	1.0-4.0	4.0-52.0	3-42.3	0.49-9.89	0.03-8.0	0.4-7.36	0.15-5.80	0.60-10.20	0.60-10.0	4.34-55.74	3.0-40.9
Mean	1.08	0.90	3.27	2.79	17.28	14.47	3.43	1.93	1.57	1.59	2.76	3.55	20.95	14.59

A * Before monsoon B ** After monsoon

Table 2. Seasonal variations in characteristics of underground irrigation waters of Bikaner district

Characters	EC (dSmt)		pH		SAR		RSC (me L ⁻¹)	
	A *	B **	A	B	A	B	A	B
Range	0.67-6.52	0.49-5.28	7.30-8.78	7.75-8.90	4.85-34.65	3.16-52.16	Nil-5.50	Nil-3.00
Mean	2.58	2.02	8.30	8.44	14.84	9.77	1.30	0.44

A * Before monsoon B ** After monsoon Nil-Negative RSC values

Table 3. Physico-chemical and fertility parameters of soils of Bikaner district

Characters	Depth (cm)	Bulk density (Mg m ⁻³)	Permeability index	EC (dSm ⁻¹)	pH	ESP	Dehydrogenase activity (ug TPF /g soil)	Available nitrogen (Kg/ha)	Available Phosphorus (p ₂ O ₅ , kg /ha)	Available Potassium (k ₂ O kg/ha)
Range	0-15	1.38-1.60	54.70-136.84	0.10-1.15	8.57-9.21	0.37-4.35	0.90-10.12	62.76-370.85	3.25-62.31	108.78-372.54
	15-30	1.40-1.63	56.08-156.82	0.08-0.95	8.62-9.28	0.66-4.75	0.46-8.33	56.95-307.40	2.20-53.13	100.79-357.50
Mean	0-15	1.49	89.30	0.42	8.74	2.19	4.25	152.82	17.51	209.99
	15-30	1.52	96.16	0.37	8.80	2.42	3.48	127.75	15.16	189.26

Table 4. salt regime constant (d) as related to salt and water characteristics of selected villages of Bikaner district of Rajasthan during 2004

Sample Code	ECe (dSm ⁻¹) during April-May-2004 (a)	ECe (dSm ⁻¹) during Oct. 2004 (b)	EC _e (dSm ⁻¹) (c)	Total rain water (mm) (v)	Soil depth (m) (m)	Bulk density (Mg m ⁻³) (ds)	Salt regime constant (g 100/g soil) (d)
Pal ₂	3.79	2.76	0.01	114	0.30	1.56	-0.0150
Kil ₂	3.25	1.92	0.01	114	0.30	1.48	-0.0210
Bam ₂	4.18	2.67	0.01	114	0.30	1.57	-0.0220
Pem ₁	6.21	4.83	0.01	114	0.30	1.54	-0.0200
Pem ₂	5.06	3.81	0.01	114	0.30	1.55	-0.0180
Sud ₁	1.80	1.37	0.01	181	0.30	1.54	-0.0070
San ₁	2.37	1.86	0.01	181	0.30	1.53	-0.0080
Jet ₁	23.23	13.60	0.01	181	0.30	1.62	-0.1310
Thu ₁	4.22	2.96	0.01	181	0.30	1.58	-0.0180
Aka ₃	3.63	2.02	0.01	123	0.30	1.49	-0.0260
Jha ₃	17.10	11.97	0.01	123	0.30	1.51	-0.0810
Cha ₁	5.31	3.83	0.01	123	0.30	1.45	-0.0240
Kot	6.33	4.42	0.01	123	0.30	1.53	-0.0290
Mul ₁	1.40	0.90	0.01	182	0.30	1.48	-0.0078
She ₁	7.09	4.16	0.01	182	0.30	1.49	-0.0460
Kho ₁	5.78	4.09	0.01	182	0.30	1.45	-0.0280
Dhap ₁	7.00	4.04	0.01	182	0.30	1.51	-0.0460
Muk ₁	20.20	17.45	0.01	222	0.30	1.49	-0.0420
Muk ₂	7.48	4.05	0.01	222	0.30	1.57	-0.0500
Ras	6.06	2.75	0.01	222	0.30	1.44	-0.0540

Efficacy of different fungicides against *Ascochyta pisi* causing Ascochyta blight of pea under laboratory conditions

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Pea occupies an important position amongst the vegetable crops of India and is one of the few crops that can be successfully grown during the winter season in India. However, Ascochyta blight caused by *Ascochyta pisi* Lib. is responsible for substantial yield losses in pea to the tune of 30-75 per cent under favourable conditions for disease development (Roger and Tivoli, 1996). The disease is polycyclic and can develop rapidly during periods of wet weather and moderate temperatures. Since the pathogen survives mainly through seeds (Kaiser, 1972). In the present investigation, the efficacy of the fungicides against the pathogen *Ascochyta pisi* under laboratory conditions was studied.

Isolation made from diseased leaf samples on 2% PDA medium yielded *Ascochyta pisi* during Rabi 2005-06. Efficacy of fungicides against *Ascochyta pisi* fungus was done by poison food technique under laboratory conditions at Department of Plant Pathology, Faculty of Agriculture and Regional Research Station (SKUAST-K), Wadura, Sopore. Three replications were maintained with three doses for each treatment. Mycelial disc of 5 mm dia. from 7 days old fungal culture of *Ascochyta pisi* was placed in the centre of each Petri plate containing 2% PDA medium incorporated with different fungicides. The medium without fungicide served as control. These petriplates were incubated at 25 °C for 7 days. The diameter of test fungus was measured. The per-cent inhibition of mycelial growth was calculated using the formula given by Vincent (1947).

$$PDI = \frac{\text{Growth in control} - \text{Growth in treatment}}{\text{Growth in control}} \times 100$$

The data in Table I revealed that all the fungicides significantly inhibited mycelial growth of the fungus *Ascochyta pisi* under laboratory conditions. Benomyl was found most effective fungicide against *Ascochyta pisi*. It inhibited 100% mycelial growth of this fungus at all three

doses viz, 400, 500 and 600 ppm followed by carbendazim which also gave 100% mycelial growth inhibition at only two doses viz, 500 and 600 ppm but 95.54% mycelial growth inhibition at 400 ppm. Next best fungicides were diniconazole, hexaconazole and thiram which also effective against the pathogen. Least effective fungicide was ziram which gave 21.66% fungal growth inhibition at 1000 ppm.

Bashir and Ilyas (1983) reported that benomyl, daconil and topsin -M were found most effective under laboratory conditions in inhibiting mycelial growth and spore germination of *Ascochyta rabiei* causing Ascochyta blight of chickpea. Khan and Khan (2006) also found that benomyl showed 100% inhibition in radial mycelial growth of *Macrophomina phaseolina* causing Macrophomina leaf spot of mung bean. Srivastava and Mishra (2008) observed that 100% inhibition in mycelial growth of *Colletotrichum truncatum* (leaf blight of urd bean) by carbendazim, benlate and hexaconazole.

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Table 1. Inhibitory effect of fungicides on the mycelial growth of *Ascochyta pisi* under laboratory Conditions

Treatment	Concentration (ppm)	Mean of mycelial growth (mm)	% Inhibition over control
Benomyl	600	0.0 (1.00)*	100.0
	500	0.0 (1.00)	100.0
	400	0.0 (1.00)	100.0
Carbendazim	600	0.0 (1.00)	100.0
	500	0.0 (1.00)	100.0
	400	3.5 (2.12)	95.54
Diniconazole	400	0.0 (1.00)	100.0
	300	0.0 (1.00)	100.0
	200	4.5 (2.34)	94.27
Hexaconazole	400	0.0 (1.00)	100.0
	300	0.0 (1.00)	100.0
	200	5.2 (2.49)	93.38
Thiram	3000	0.0 (1.00)	100.0
	2000	0.0 (1.00)	100.0
	1000	8.5 (3.08)	89.17
Captan	4000	0.0 (1.00)	100.0
	3000	0.0 (1.00)	100.0
	2000	7.5 (2.92)	90.45
Dodine	700	21.5 (4.74)	72.61
	600	26.0 (5.19)	66.88
	500	34.5 (5.96)	56.05
Copper oxychloride	3000	20.5 (4.64)	73.89
	2000	29.0 (5.48)	63.06
	1000	39.5 (6.36)	49.68
Mancozeb	4000	23.0 (4.90)	70.70
	3000	34.5 (5.96)	56.05
	2000	47.5 (6.96)	39.49
Zineb	3000	23.5 (4.95)	70.06
	2000	35.5 (6.04)	54.78
	1000	48.0 (6.98)	38.85
Ziram	3000	28.5 (5.34)	63.49
	2000	47.0 (6.93)	40.13
	1000	61.5 (7.91)	21.66
Control	-	78.5 (8.92)	-
CD		(0.85)	

* Figures in parenthesis are square root transformed values.

Performance of Ashgourd genotype under Hyderabad conditions of Andhra Pradesh

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Ashgourd [*Benincasa hispida* (Thumb) Cogn.] is an important warm vegetable grown for its fruit used in confectionery and ayurvedic medicinal preparations (Indira and Peter, 1987). Among the cucurbitaceous vegetable, where as ripe fruit is boiled in sugar to make a confectionery known as petha (Joydip Mandal *et. al.* 2002). In spite of its economic importance, very little attempts has been made so far to improve the crop production. The genotypes under cultivation are nondescript ones. Poor genetic stock, inadequate and improper management practices and incidence of many parasitic diseases are main constraints for low yield. To overcome this, development of early maturing and high yielding varieties/hybrid is most urgent need for the farmers. Hence, this experiment was conducted to identify the suitable varieties to this area. Since, there is a continuous demand for new high yielding varieties, genotypes developed at various centers under All India Coordinated Research Project (AICRP) and at vegetable Breeding Station, Agricultural Research Institute, Rajendranagar, Hyderabad were evaluated to identify the suitable genotypes for this area.

The experiment was conducted at Vegetable Breeding Station, Agricultural Research Institute, Rajendranagar, Hyderabad during Kharif 2005-2007 in clay loam soil to evaluate the material developed at ICAR stations, State Agricultural Universities for their suitability in Andhra Pradesh. The agro climate of Hyderabad is characterized by minimum temperature 20.3°C, maximum temperature 32.6°C, average rainfall 733 mm and humidity of 63.3%. The experiment was laid out in Randomized Block Design with 4 replications having plot of 5 m x 2.5 m with adopted spacing 5 m x 0.5 m. observations were recorded on vine growth, number of lateral/vine, node number at which first female flowers appeared, number of fruits /vine, average fruit weight, fruit length, diameter and yield per hectare. The crop was raised as per the recommended cultivation practices during the season. The data was statistically analyzed as described by Panse and Sukhatme (1987).

Table 1. Preliminary information on five genotypes in Ashgourd under study.

S.No	Name of the Entry	Source
1	HYBH-24	ANGRAU, Hyderabad
2	HYBH-24	ANGRAU, Hyderabad
3	BH-21	Vellanikkara
4	Pusa Ujjawal	IARI
5	Shakti	ANGRAU, Hyderabad

The preliminary information on five genotypes of Ashgourd under study is given in table 1. The results revealed that the mean node number to first female flower appeared ranged from 9.8 to 11.5 (Table-2). It was found that the appearance of first female flower was on the lowest node in HYBH-25 (9.8) followed by local Check Shakti (10.2). The trait such as days to appearance of first female flowering and node number at which first female flower appears are most useful parameters indicating earliness of genotype (Samadia, 2002). Number of female flowers and fruit per plant are the major deciding factors to assess the yield potential of genotypes. The genotypes with more number of female flowers at earlier nodes along with early fruit setting and picking should result in higher and early production. In the present study, the number of fruits/vine ranged from 1.0 to 1.4. The check Shakti recorded significantly highest fruit number than all the genotypes under study. The average fruit weight at maturity stage varied from number than all the genotype under study. The average fruit weight at maturity stage varied from 2.7 kg to 4.4 kg. Genotype HYBH-25 recorded significantly highest average fruit weight (4.4 kg) followed by HYBH-24 (4.2 kg) than other genotypes. In Ashgourd medium sized fruits are preferred for domestic purpose than large sized fruits. Marketable yield ranged from 3.2 kg to 5.2 kg/vine. Among the genotypes, HYBH-24 recorded significantly highest fruit yield/vine (5.2 kg) which is on par with Shakti (4.6 kg) and HYBH-25 (4.4 kg). Low yield was observed in Bh-21 (3.2 kg) per vine. Similar results were reported at Indian Institute of Vegetable Research, Varanasi (Anonymous 2006-2007). Performance of growth and plant vigour revealed that there was no significant difference in terms of vine length and number of laterals/vine.

These results suggested that genotypes HYBH-24 and HYBH-25 developed at Vegetable Breeding Station,

Table 2. Morphometric and yield characters of ash gourd genotypes.

Genotypes	Vine Length (m)	No. of laterals/vine	Node at 1 st female flower	Average fruit wt. (kg)	No. of fruits /vine	Fruit length (cm)	Fruit diameter (cm)	Yield kg/vine	Yield t/ha
HYBH-24	6.0	2.3	11.5	4.5	1.5	56.5	60.5	6.5	25.9
HYBH-25	5.9	2.3	9.8	4.2	1.2	58.7	61.9	4.8	19.1
Pusa Ujjwal	6.6	2.2	10.6	3.3	1.1	53.2	50.7	3.5	14.1
Bh-21	6.4	2.3	10.5	3.0	1.3	56.4	51.5	3.8	15.0
Shakti(c)	5.6	2.3	10.2	3.3	1.5	54.0	55.9	5.1	20.2
SFm±	0.2	0.1	0.3	0.2	0.1	2.7	2.9	0.4	1.6
C.D. at 5%	0.6	NS	0.9	0.6	0.2	NS	NS	1.2	4.9

Agricultural Research Institute, Rajendranagar, Hyderabad can be grown in and around Hyderabad, Andhra Pradesh as they are performed better compared to other genotypes.

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PCR compatible, genomic DNA extraction procedure
for *lasora* (*Cordia myxa* (Roxb.))P.N. Sivalingam¹ and Dhurendra Singh

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Cordia myxa (Roxb.) is commonly known as "lasora". It is an under-exploited fruit and herbal tree in rural India. It belongs to the family *Boraginaceae* and probably originated in the Indian sub-continent (Samadia, 2005). It is widely distributed in the arid and semi-arid regions of India and yields fruit during April-May. Immature fruits of *lasora* are used for making pickles and vegetable curries, and matured fruits are very nutritious, rich in carbohydrates and total ash. This fruit tree is not a commercial crop but it grows naturally in the non-cultivable or waste lands, backyards, on roadsides and farm boundaries (Samadia, 2005). Conservation and understanding genetic diversity of this under-exploited crop is the primary requirement for identification of superior genotypes for its improvement and exploitation. Considerable diversity on the basis of morphological parameters has been reported in the naturally growing population of *lasora* in Rajasthan (Samadia, 2007). No report is available on the genetic diversity of *lasora* germplasm using DNA markers. Good quality, PCR compatible genomic DNA is essential for most of the PCR based markers. Our experience showed that the genomic DNA extracted from leaf tissue of *lasora* using many commercial kits did not yield amplicons with random hexaprimers (RAPD primers) in PCR (unpublished data). Therefore, standardization of a PCR-compatible DNA extraction procedure is essential for *lasora*. Here, we report the standardized PCR-compatible genomic DNA extraction procedure from the leaf tissue of *lasora*.

Young leaf tissues (2nd leaf from top of the twig) were collected from a single *lasora* tree and stored in an ice-box and brought to the laboratory immediately. One hundred mg of leaf tissue was weighed and stored at -20°C from eighteen samples (three samples for each treatment) (Table 1). Each sample was ground separately in a pestle and mortar with liquid nitrogen and transferred to 1.5 ml microcentrifuge tube. One ml of DNA extraction buffer (Dellaport *et al.*, 1983) (Table 1) was added to three samples separately and processed. These samples were incubated at 65 °C in a water bath for one hour. Samples were then

removed from the water bath and brought to room temperature. To remove protein and other organics, 0.6 volume of chloroform: isoamyl alcohol (24:1) was added to the sample and mixed for 20 minutes and centrifuged at 10000 rpm for 10 minutes at 4 °C. The supernatant was removed and transferred to a fresh microcentrifuge tube and again chloroform: isoamylalcohol (24:1) was added, mixed and centrifuged at 10000 rpm for 10 minutes at 4 °C. The supernatant was removed and transferred to a fresh microcentrifuge tube. To the supernatant, 0.8 volume of isopropanol was added and mixed gently and centrifuged at 10000 rpm for 10 minutes at 4 °C. The supernatant was decanted and to the pellet 400 µl of 70 % ethanol was added and centrifuged for 10 minutes. The supernatant was decanted and the pellet was dried at room temperature for 30 minutes. 100 µl of sterile double distilled water was added to the pellet and allowed to dissolve for two hours at room temperature or overnight at 4 °C. After dissolving, an aliquot of 50 µl was taken from each sample and transferred to a fresh micro-centrifuge tube. Two µl of RNase A (10mg/ml) (MBI Fermentas) was added to each sample and kept at 37 °C for 30 minutes. The volume in each sample was made up to 200 µl with sterile double distilled water. Equal volume of chloroform: isoamyl alcohol (24:1) was added, mixed and centrifuged as described above and the supernatant was removed and transferred to a fresh microcentrifuge tube. To the supernatant, 1/10th volume of sodium acetate (pH 4.8) followed by 0.8 volume of isopropanol were added and mixed gently. The subsequent steps are same as described previously.

Four µl from each tube was loaded on a 0.7 % agarose gel with 10 µl sterile double distilled water and 2 µl of 6x loading dye and electrophoresed. After electrophoresis the gel was viewed under a UV transilluminator to check the quality of DNA. Quantification of DNA was done spectrophotometrically. The genomic DNA extracted from *lasora* was used in PCR to check its compatibility. A 50 as well as 25 µl PCR reaction volume was set up containing 100 ng of template DNA, 3mM MgCl₂, 20 pmol random primer (OPBE 07, Operon Technologies, USA), 1U *Taq* DNA polymerase, 0.2mM dNTP (MBI Fermentas) with the following thermal profile: initial denaturation of 94°C for 5

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Table 1 Effect of different treatments on DNA yield, quality and its compatibility in PCR

S.No.	Buffer details	DNA Yield µg/ µl	Quality of DNA	Compatibility in PCR*
Without RNase A treatment				
1.	DNA extraction buffer only	0.40-0.45	Sheared DNA with degraded RNA	Yes
2.	DNA extraction buffer + 0.1% β-ME	0.45-0.48	Less sheared DNA with degraded RNA	Yes
3.	DNA extraction buffer + 0.5% β-ME	0.75-0.80	No shearing of DNA with degraded RNA	Yes
4.	DNA extraction buffer + 1.0% β-ME	0.75-0.80	No shearing of DNA with degraded RNA	Yes
5.	DNA extraction buffer + 0.1% SDS	0.60-0.63	No shearing of DNA with degraded RNA	Yes
6.	DNA extraction buffer + 1% PVP	0.65-0.70	No shearing of DNA with degraded RNA	Yes
With RNase A treatment				
7.	DNA extraction buffer only	0.35-4.00	Sheared DNA, no RNA	Yes
8.	DNA extraction buffer + 0.1% β-ME	0.44-0.46	Less sheared DNA, no RNA	Yes
9.	DNA extraction buffer + 0.5% β-ME	0.72-0.77	No shearing of DNA, no RNA	Yes
10.	DNA extraction buffer + 1.0% β-ME	0.71-0.75	No shearing of DNA, no RNA	Yes
11.	DNA extraction buffer + 0.1% SDS	0.52-0.64	No shearing of DNA, no RNA	Yes
12.	DNA extraction buffer + 1% PVP	0.60-0.63	No shearing of DNA, no RNA	Yes

DNA extraction buffer- 100mM Tris-HCl (pH 8.0) + 1.4 M NaCl + 20mM EDTA + 2% CTAB

* DNA yield measured at 260 nm through spectrophotometer

** PCR compatibility checked with RAPD primer, OPBE-7

β-ME: β-mercaptoethanol; SDS: Sodium dodecyl sulphate; PVP: Polyvinyl pyrrolidone

min followed by 35 cycles of 94°C for 30 seconds, 36°C for 1 min and 72°C for 2 min 30 seconds. Final extension was set at 72°C for 10 min. The PCR products were loaded on a 1.4 % agarose gel pre-stained with ethidium bromide, electrophoresed and viewed under a UV transilluminator. The standardized DNA extraction and PCR procedure using random primers were validated with 22 *lasora* genotypes available at the experimental farm of Central Institute for Arid Horticulture.

On the basis of different treatments in the extraction of PCR compatible genomic DNA from *lasora*, higher amounts of DNA (0.75-0.80 µg/µl) was obtained with DNA extraction buffer containing 0.5 or 1.0 % β-ME without RNase A treatment followed by additives like SDS or PVP (Table 1). Similarly, the quality of DNA was also better in the β-ME treated samples. The quality of DNA in these samples was equally good even after RNase treatment, except for a slight reduction in the yield of DNA (~ 0.5 µg/µl). Though, the yield and quality of DNA varied with different additives used with DNA extraction buffer, all the DNA samples with or without RNase treatment were found

to be PCR compatible. The results obtained indicated that the quality of DNA may be improved by DNA extraction buffer alone which could easily remove the PCR inhibitors present in the leaf tissue. The *lasora* leaves contain high polysaccharides/mucilaginous substances (Samadia, 2005) and phenolics which inhibit *Taq* DNA polymerase activity (Fang *et al.*, 1992) in PCR. PCR incompatibility of genomic DNA extracted through commercial kits might have been due to the ineffective removal of inhibitors. However, this problem has been addressed by our current method. It was observed that the additives used in the present study could improve the DNA yield greatly. DNA extracted using 0.5 to 1.0 % β-ME added to the extraction buffer and without RNase A treatment was found superior to all other treatments. The reason for higher DNA yields may be due to the fact that β-ME is a reducing agent and it probably inhibited the activity of DNA degrading nucleases (Lodhi *et al.*, 1994). Among the treatments, DNA extraction buffer with 0.5% β-ME without RNase treatment was found to be the best for DNA extraction and 25 µl of final volume in PCR is sufficient. Genomic DNA extracted from 22

genotypes of *lasora* available at the CIAH farm using this procedure was found PCR compatible with different RAPD primers. This procedure could be very useful in studying its genetic diversity through DNA based markers and also for its improvement. To our knowledge, this is the first report on a DNA extraction procedure for the mucilaginous fruit tree, *lasora*.

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Effect of plant growth substances on growth and flowering attributes of African marigold (*Tagetes erecta* Linn.)

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Floriculture is now emerging as an important venture in the world, especially as a potential money spinner. Many flowers and ornamental plants grown for domestic as well as for export market provide more returns per unit area than other horticultural crops. Marigold (*Tagetes sp.*) is an annual flower to cultivate easily and have wide adaptability to different soil and climatic conditions. It belongs to family Compositae and native of India. It has a great economic potential for loose flower trade. It is especially used for making garland, decoration and induced in landscape plans due to its variable height and colour. Marigolds have also great medicinal importance i.e. leaf extract is good remedy for ear-ache and flower extract is used as blood purifier and against bleeding piles. Besides, it is also found important in controlling nematodes because, it produces thiophenes, which are naturally occurring biocides. Application of plant growth substances showed tremendous potential in modulating growth and related processes. They are considered bio-soft wards, which elicit rapid as well as long term responses in crop plants (Yadav *et al.*, 1994 and Malik *et al.*, 1996). NAA is an important growth substance, which stimulates the cell division, cell enlargement and cell elongation in apical region. BA and kinetin are cytokinin which has been found essential for growth and development of plant organs, retention of chlorophyll, translocation of nutrient (Pandey and Sinha, 1986). Considering the importance of plant growth substances, to find out the best growth substance and its concentration in response of growth and flowering of African marigold.

The experiment was undertaken on African marigold (*Tagetes erecta* Linn.) var. Cracker Jack" at Horticultural Farm, Department of Horticulture, SKN College of Agriculture, Jobner. The soil of experimental plot was loamy sand with 8.10 pH and 1.20 dSm⁻¹ EC at 25°C. The experiment was laid out in a RBD (Randomized Block Design) with three replications. The randomization of the treatments was done

with the help of random number table. Crop was raised during rabi season of 2009-10 in 2.4 x 1.8 m² plot size maintaining 60 x 45 cm spacing. The treatments comprised of three levels each of NAA (100, 200, 300 ppm), BA (25, 50, 75 ppm) and kinetin (50, 100, 150 ppm) thus, making 10 treatments including absolute control. For preparation of solution, 1N NaOH with fresh water (Chawla, 2009) was used as sticking agent. Two successive spray of these growth substances was done at 30 and 45 DAT (days after transplanting) of seedlings. All essential cultural practices were followed to maintain optimum plant stand. The mean plot data recorded on five randomly selected plants on growth and flowering traits were subjected to statistical analysis (Panse and Sukhatme, 1995).

The application of growth substances brought perceptible variation in plant growth and flowering attributes of marigold as compared to control (Table 1). Spray of NAA had maximum plant height (40.82, 73.95 cm), number of branches/plant (10.59, 13.93) and plant spread (32.64 x 29.51, 58.31 x 54.62 cm) at 45 and 90 DAT, respectively. Number of flower per plant (55.04) and longevity of intact flowers (17.29 days) also recorded maximum with NAA application. This increase in plant growth and flowering with the application of NAA might be due to active cell division, cell elongation, cell enlargement and osmotic uptake of water and nutrients (Pandey and Sinha, 1984). These results are in close conformity with those of Kumar *et al.* (2008) in gladiolus, Mukhopadhyay and Mukhopadhyay (1990) in Carnation and Singh and Kumar (2003) in Rose. The application of kinetin had more pronounced effect on number of days taken to first flower appearance was recorded lowest with kinetin (58.66 DAT) as compared to NAA and BA application. Therefore, kinetin had maximum number of pickings (8.10), which was significantly higher over NAA and statistically at par with BA. This change in first flowering appearance and number of pickings was might be due to cell division and cell elongation promoted by cytokinin. These results have also been in consonance with Sonvir *et al.* (2002) and Kim *et al.* (2003).

The increasing concentrations of plant growth

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Table: 1 Effect of PGSSs on growth and flowering attributes of African marigold

Treatments	Plant height (cm)		Number of branches/plant				Plant spread (cm ²)				Days taken to flowering	Number of flowers per plant	Number of pickings	Longevity of intact flower (days)
	45	90	45	90	45	90	45 DAT	N-S	E-W	90 DAT				
	DAT	DAT	DAT	DAT	DAT	DAT								
Control	33.66	64.40	7.86	10.93	26.93	6.81	48.53	45.66	67.66	41.93	61.33	13.66		
Rest	38.97	71.51	9.93	13.02	31.04	7.73	56.11	52.17	60.44	51.81	69.41	16.29		
SEm+	1.63	3.02	0.48	0.55	1.32	0.31	2.27	2.11	2.50	2.10	2.80	0.66		
CD	4.63	NS	1.36	1.56	3.73	0.88	6.43	5.99	7.09	5.94	7.92	1.87		
NAA	40.82	73.95	10.59	13.93	32.64	7.32	58.31	54.62	62.33	55.04	65.23	17.29		
BA	36.49	69.68	8.82	12.11	29.28	7.77	52.75	49.51	60.33	45.37	70.00	15.17		
Kinetin	39.62	70.89	10.37	13.02	31.19	8.10	57.26	52.40	58.66	55.02	73.00	16.42		
SEm+	0.77	1.42	0.23	0.26	0.62	0.15	1.07	1.00	1.18	0.99	1.32	0.31		
CD	2.18	4.04	0.64	0.74	1.76	0.41	3.03	2.82	3.34	2.80	3.73	0.88		

(p=0.05)

(p=0.05)

NS=Non-significant, N-S=North-South, E-W=East-West, DAT=Days after transplanting

Table : 2 Effect of various concentrations of PGs on growth and flowering attributes of African marigold

atments	Plant height (cm)		Number of branches/plant				Plant spread (cm ²)				Days taken to flowering	Number of flowers per plant	Number of pickings	Longevity of intact flower (days)
	45		90		45		90							
	DAT	DAT	DAT	DAT	N-S	E-W	N-S	E-W						
A 100 ppm	38.06	70.00	9.66	13.20	31.33	7.00	55.46	51.86	65.33	52.40	63.00	16.40		
A 200 ppm	41.13	74.60	10.86	14.06	32.80	7.37	58.66	55.13	62.66	55.13	64.35	17.46		
A 300 ppm	43.26	77.26	11.26	14.53	33.80	7.59	60.80	56.86	59.00	57.60	68.33	18.00		
n _±	1.33	2.47	0.39	0.45	1.07	0.25	1.85	1.73	2.04	1.71	2.28	0.54		
(p=0.05)	3.78	6.99	1.11	1.28	NS	0.72	5.25	4.89	5.79	4.85	6.47	1.52		
25 ppm	34.33	65.06	8.13	10.66	27.86	7.33	50.00	46.80	63.66	42.18	66.00	14.26		
50 ppm	36.73	69.66	8.93	12.60	29.46	7.88	52.93	49.73	60.00	45.76	71.00	15.33		
75 ppm	38.40	74.33	9.40	13.06	30.53	8.11	55.33	52.00	57.33	48.16	73.00	15.93		
n _±	1.33	2.47	0.39	0.45	1.07	0.25	1.85	1.73	2.04	1.71	2.28	0.54		
(p=0.05)	3.78	6.99	1.11	1.28	NS	0.72	5.25	4.89	5.79	4.85	6.47	1.52		
etin 50 ppm	37.86	67.73	9.73	12.60	30.26	7.66	54.73	49.80	62.00	51.97	69.00	15.80		
etin 100 ppm	39.60	71.00	10.66	13.00	31.26	8.14	57.66	52.73	58.33	55.86	73.33	16.60		
etin 150 ppm	41.40	73.93	10.73	13.46	32.06	8.51	59.40	54.66	55.66	57.22	76.66	16.86		
n _±	1.33	2.47	0.39	0.45	1.07	0.25	1.85	1.73	2.04	1.71	2.28	0.54		
(p=0.05)	NS	NS	NS	NS	NS	0.72	NS	NS	5.79	4.85	6.47	1.52		

NS=Non-significant, N-S=North-South, E-W=East-West, DAT=Days after transplanting

substances significantly increased growth and flowering parameter of African marigold (Table 2). NAA @ 300 ppm recorded highest plant height (43.26, 77.26 cm), number of branches/plant (11.26, 14.53) and plant spread (33.80 x 30.46, 60.80 x 56.86 cm²) at 45 and 90 days after transplanting of seedlings, respectively. NAA @ 300 ppm also recorded maximum number of flowers per plant and highest longevity of intact flower (18.00 days). The minimum number of days taken to first flower appearance (55.66 days) and highest number of pickings (8.51) was observed with kinetin @ 150 ppm. Similar results have also been obtained by Choudhary and Khandelwal (2008) in gladiolus, Singh and Kumar (2003) in rose and Singh (2003) in French marigold. Sharma *et al.* (1995) found increase in plant height of chrysanthemum with increasing levels of NAA from 25 to 100 ppm spray.

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Heterotic response in sponge gourd [*Luffa cylindrical* (Roem) L.]

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Sponge gourd [*Luffa cylindrical* (Roem) L.] plays a significant role in the diet of human nutrition, especially in tropical countries. It is one of most popular vegetable during summer and rainy seasons in northern India. Sponge gourd is a monoecious crop and cross pollination is a general rule which facilitates exploration of heterosis. It exhibits high heterosis (Mole *et al.*, 2001 and Ram *et al.* 2004) which suggest a great scope of improvement through heterosis breeding. Today hybrids are gaining popularity due to their earliness, high productivity, better quality and adaptation to environmental condition. Realizing the importance of hybrid vigour, an investigation was undertaken to identify potential parental combinations in order to have superior hybrids with good yield and quality traits.

Fifteen diverse sponge gourd inbreds viz., VRSG-1, VRSG-4, VRSG-5, VRSG-6, VRSG-8, VRSG-11, VRSG-12, VRSG-41, VRSG-42, VRSG-44, VRSG-45, VRSG-46, VRSG-49, VRSG-52, VRSG-61 were selected and crossed with three testers (VRSG-2, VRSG-3 and VRSG-66) resulting in 45 F_1 s. The F_1 s and parents were evaluated under complete randomized block design replicated thrice at IIVR Seed Production Centre, Kushinagar (UP) during rainy season of 2009. Seeds were sown keeping row to row and plant to plant spacing 3 m and 70 cm, respectively. Eight competitive plants in each treatment under each replication were recorded for node on which first female flower appeared, days to anthesis of first female flower, fruit length (cm), circumference of fruit (cm), marketable fruits per plant, early yield per plant (g) and total marketable yield per plant (g). The data were analyzed by Line x Tester analysis outlined by Kempthorne (1957) and heterosis was calculated as percentage of F_1 performance in the favourable direction of its better parent (BP) as suggested by Hayes *et al.* (1955).

The data presented in Table 1 revealed significant and desirable heterosis over BP for all the characters except circumference of fruit. For two traits namely node on which first female flower appeared and days to anthesis of first female flower negative heterosis was considered to be better, whereas for remaining five traits the positive heterosis was considered to be desirable for judging good

F_1 hybrids in sponge gourd. The results indicated that early yield per plant and total marketable yield per plant were most heterotic traits. The highest positive heterosis was recorded for early yield per plant in VRSG-1 x VRSG-66 (76.31 %) followed by total marketable yield per plant in VRSG-4 x VRSG-66 (72.84%). Only one cross combination VRSG-8 x VRSG-66 exhibited significant and positive heterosis (15.53%) for fruit length. The highest fruit yield recorded by the best performing hybrid VRSG-4 x VRSG-66 could be attributed due to increased number of marketable fruits, fruit length and circumference of fruit. The best performing hybrid for number of marketable fruits per plant was found to be VRSG-4 x VRSG-66 which showed 44.70% heterosis over BP. These findings are supported by the results of Ram *et al.* (2004) in sathputia and Mole *et al.* (2001) and Hedau *et al.* (2004) in ridge gourd.

The characters as node on which first female flower appeared and days to anthesis of first female flower for which negative heterosis is desirable was shown by the crosses VRSG-6 x VRSG-66 (-45.09%) and VRSG-5 x VRSG-2 (-22.37%), respectively. The results are also in conformity with those obtained by Ram *et al.* (2004) in sathputia and Singh *et al.* (2007) in bitter gourd. The number of significant crosses and range of heterosis based on BP are presented in Table 2. Among 45 F_1 s, only 13 cross showed overall best performance for different traits. Considering the marketable fruits per plant and total marketable fruit yield per plant the best performing hybrid is VRSG-4 x VRSG-66 which may be tested for yield and other economic traits for performance under different agro climatic conditions for the development of hybrids.

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Table 1. Top three hybrids selected separately on the basis of heterosis over better parent

S. No.	Character	Cross	Heterosis (%)
1.	Node on which first female flower appeared	VRSG-6 x VRSG-66	-45.09**
		VRSG-52 x VRSG-66	-38.30*
2.	Days to anthesis of first female flower	VRSG-52 x VRSG-2	-34.04*
		VRSG-5 x VRSG-2	-22.37**
		VRSG-46 x VRSG-66	-20.93**
3.	Fruit length (cm)	VRSG-44 x VRSG-66	-16.27**
		VRSG-8 x VRSG-66	15.53*
		VRSG-49 x VRSG-66	13.19
4.	Circumference of fruit (cm)	VRSG-41 x VRSG-66	12.59
		VRSG-5 x VRSG-3	10.91
		VRSG-41 x VRSG-66	9.65*
5.	Marketable fruits per plant	VRSG-5 x VRSG-66	7.49
		VRSG-4 x VRSG-66	44.70**
		VRSG-46 x VRSG-2	36.40*
6.	Early yield per plant (g)	VRSG-46 x VRSG-3	31.93*
		VRSG-1 x VRSG-66	76.31**
		VRSG-4 x VRSG-2	62.29**
7.	Total marketable yield per plant (g)	VRSG-5 x VRSG-2	23.23*
		VRSG-4 x VRSG-66	72.84**
		VRSG-8 x VRSG-2	48.03*
		VRSG-8 x VRSG-66	42.62*

Table 2. Mean performance and range of heterosis in sponge gour

S.No.	Characters	Mean of parents	Mean of hybrids	Range of heterosis (%)	No. of desirable and significant cross over better parent
1.	Node on which first female flower appread	15.10	13.49	-45.09 to 68.87	5
2.	Days to anthesis of first female flower	56.19	51.86	-22.37 to 6.21	7
3.	Fruit length (cm)	28.41	31.15	-8.95 to 15.52	1
4.	Circumference of fruit (cm)	6.05	6.26	-20.48 to 10.91	-
5.	Marketable fruits per plant	4.78	5.74	-36.67 to 44.70	3
6.	Early yield per plant (g)	64.01	72.39	-68.48 to 76.31	6
7.	Total marketable yield per plant (g)	404.81	531.81	-34.78 to 72.84	5