

Efficient use of drumstick in multitier cropping system under semi-arid ecosystem

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Abstract

To achieve the projected target of 220 MT of vegetable production by 2020, augmentation in vegetable production needs to be formulated for different cropping system to fully exploit the natural resources. Number of cropping systems have been developed for different ecosystems, however, a suitable vegetable based cropping system is lacking for semi-arid and arid ecosystem. Considering the nature of growth pattern of the component crops and their yield potential, drumstick (base crop) and cucurbits (climbers) have been chosen in the cropping system. An experiment comprising eleven treatments with three replications in randomized block design was conducted at Central Horticultural Experiment station, Vejalpur from 2004-05 and 2005-06. The results revealed that the interaction of component crops did not significantly reduce the growth parameters of base crop (drumstick) over sole crop. Higher the cropping index (200%), drumstick equivalent yield (28.65 kg/ha) and land equivalent ratio (2.39) has been observed in the treatment combining drumstick and ridge gourd. This multitier system reduced the cost of input by 70.1 and 64.0 per cent comparing bower system and local system respectively.

Key words: Drumstick, cropping system, cucurbits

Introduction

In India, the current status on vegetable production (94.0 MT) is capable to supply only 175 g vegetables per capita per day against the recommendation by dietician (300 g). To fulfill the recommended consumption level, 220 MT of vegetables required to be produced by 2020 to the projected population of 1.5 billion. The threat on shrinking of agricultural land, ignited an opportunity for diversification towards plant architectural management and utilization to fit them into the cropping system approach. The present concept of cropping system defines the yearly sequences and spatial arrangements of crops (horizontal approach) and its interaction with farm resources. The plant architectural arrangement and utilization (vertical approach) in the cropping system has been completely neglected so far.

Suitability of component crops depends primarily on soil and climatic conditions, however, compatibility aspects deserve prime consideration. Competition of crops for external factors (light, space, nutrients) and internal factors (flowering and fruiting period) leads to reduce yield and poor suitability in the cropping system. Hence, selection of compatible crops and their planting geometry could increase the productivity and net income (Shivaramu and Shivshankar, 1992). Considering the nature of growth pattern of the component crops and their yield potential, drumstick (base crop) and cucurbits (climbers or supportive

crops) are the vegetables which are highly grown by the poor farm holdings as pure crops. Presently, drumstick is the most widely known vegetables for its drought tolerant (Palada, 1996) highly nutritious vegetable (Ramachandran, 1980) grown at about 380.5 km² producing 1.1 to 1.3 million tonnes of tender fruits (Rajangam *et al.*, 2001). Presently, drumstick is getting momentum in northern parts of India too. Cucurbitaceous crops like bitter gourd, bottle gourd, ridge gourd, sponge gourd and snake gourd are cultivated at an area of 2.42 lakh ha, with production of 25.0 lakh t and productivity of 10.024 t/ha in our country (Siddhu, 2002). However, requirement of pandal for its cultivation is a greater hurdle for enhancing the area under cucurbits to the farmers, as it requires high investment.

Presently, the interspaces between the base crop has been given priority, but the plant architecture can also be utilized in the cropping system. Though, number of cropping systems has been developed for different ecosystems but they showed poor success in arid and semi-arid ecosystem due to the harsh climate and poor economic status of farming community. Hence, modified cropping system using through plant architectural management and utilization for developing a suitable vegetable based cropping system for semi-arid and arid ecosystem is a prime requisite. Hence, the present experiment was attempted to assess the suitability of drumstick under multitier cropping system, to analyze the impact of drumstick based cropping system on monetary returns, and to assess the different existing cropping systems with drumstick based system for economy and profitability.

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Materials and methods

A field experiment was conducted at Central Horticultural Experiment Station (CIAH) Vejalpur during 2004-05 and 2005-06. The experimental site is located at 22° 41' 33" - and 73° 33' 22" - and lies between 110-115m above mean sea level. The annual rainfall mainly is confined to three months (July to September) with an average of 35 rainy days a year. The annual maximum and minimum temperature ranged from 42-43°C in May and 6-7°C in January, respectively. The annual potential evapo-transpiration ranged from 1500-1600 mm against the annual precipitation of 750 mm. The soil having pH of (6.9-7.22) EC-0.1-0.2DSM, and available N-112.5-207kg/ha, P-6.75-22.05kg/ha, K-139.5-253.125 Kg/ha. The treatment details are viz. T1=drumstick (pure crop), T2=bottle gourd, T3=bitter gourd, T4=ridge gourd, T5= sponge gourd, T6= snake gourd, T7= drumstick + bottle gourd, T8= drumstick + bitter gourd, T9=drumstick + ridge gourd, T10=drumstick + sponge gourd, T11= drumstick + snake gourd. Each treatment was maintained at 5.0 x 3.0 m plot. The treatments were laid out in randomized block design and the treatments were replicated thrice. Cucurbits were sown in the second week of June and nine and four hills (around three year old drumstick) were maintained in pure and intercropping plots respectively. After establishment of vines, were directed towards drumstick trunk so as to climb on and they were strictly maintained at harvestable height. Except irrigation, all other recommended package of practices was followed to assess the potential yield of crops under sole crop and half the dose of recommended fertilizers was applied to the intercrop treatments. The observations on vegetative, floral, fruiting and yield parameters were recorded and the average was used for the statistical analysis. The drumstick equivalent yield was calculated by multiplying the yield of a particular intercrop to the market rate of that crop and divided by the drumstick market rate and finally add the drumstick yield with that figure.

The productivity of the intercropping was evaluated by the land equivalent ratio (LER) and economic net return. LER is often been considered to be an index of intercropping advantages, The LER defined as $LA+LB=A/A+B/B$, where as LA, LB are the individual LER of A and B, LA is obtained by dividing the yield of crop A in intercropping (A) by the yield of the same crop in sole cropping (AS). LB was calculated in the same (Vandermeer, 1989). Economic net income analysis was undertaken to assess economic feasibility of different intercrops.

Results and discussion

Growth attributes

Plant vigour and earliness are the prime parameters to be considered for gourds cultivation under semi-arid and arid ecosystem. Plant length, days to female flowering and fruiting in different gourd were observed and

analyzed. The data presented in Table-I clearly indicated that there was no significant different in these parameters of supportive crops on COD method than pure crop (G). Ridge gourd and sponge gourd recorded the highest plant length (more than 4.0 m) followed by bottle gourd and snake gourd (more than 3.0 m). The lowest plant length was recorded in bitter gourd (less than 2.5 m). Earliness flowering is an economically important parameters for gourds. Among the gourds tested, bitter gourd proved to record the earliest female flowering, followed by ridge gourd and sponge gourd. Snake gourd took more periods for female flowering. Further, it is understood from the non significant difference between the G and COD methods indicating suitability of drumstick as support. This might be due to the compatibility of drumstick in terms of light provided to the supportive crops growth and development. The differential behavior of components crops in intercropping system is concordance with finding of Kulbir singh *et al.* (2001)

Yield attributes

It was observed that among the supportive crops tested for yield, the highest yield was obtained in ridge gourd (48.83 kg) followed by bottle gourd (43.96 kg) under the COD method than 40.62 and 32.29 kg respectively in G method. The lowest yield was recorded in bitter gourd in both the methods. Assessing the feasibility of yield among G and COD, showed that the higher values over G method proved an increment of 44.96, 36.15 and 38.70 per cent in ridge gourd, bottle gourd, and snake gourd indicating the need of support for harvesting higher marketable produce over G method. This result is in concordance with the finding of Abuselaha and Dutta (1995) who reported that significant difference in the yield of gourds grown on 3 m height trellis (33.41%) and bower system (36%) over ground trailing method. This finding is in contrary with findings of Palada (1996) who observed that drumstick trees are highly competitive with eggplant and sweet corn and reduced yield up to 50 per cent. However, the significant improvement in the yield of gourds strongly justified from the fact that until July end, there was no dense vegetative shoot found on drumstick trees. The open centered barren branches might have facilitated sufficient sunlight to under storied crops. The non-coincidence of flowering of drumstick indicating its fitness to multitier cropping system. Fukai and Trenbath (1993) also reported that intercropping is most productive when inter crops differ greatly in growth duration so that their minimum requirements for growth resources occur at different times. Further, it was observed that when the fruit development started in drumstick trees (November), the gourds finished their life cycle under semi-arid ecosystem. However, a meager yield reduction in drumstick at 11.71, 9.74, and 8.29 per cent observed with combination of snake gourd followed bitter gourd and ridge gourd indicating the thorough study on cropping system based nutritional supplementation to avert this reduction. These

Table 1. Effect of drumstick in multitier cropping system on vegetative and flowering parameters of different cucurbitaceous crops

Cropping system	Plant length (cm)			Days to female flowering			Yield per plot (Kg)		
	2004-05	2005-06	Mean	2004-05	2005-06	Mean	2004-05	2005-06	Mean
1. Bottle gourd	246.5	238.4	242.45	56.2	57.2	56.73	30.21	34.33	32.29
2. Bitter gourd	290.1	288.3	289.23	49.2	51.3	50.25	16.39	20.15	18.20
3. Ridge gourd	473.1	459.3	466.21	56.8	58.1	57.45	38.26	43.12	40.62
4. Sponge gourd	483.5	479.1	481.32	54.2	51.5	52.85	26.10	34.51	30.28
5. Snake gourd	342.8	253.2	298.05	65.3	64.2	64.75	19.32	23.53	21.41
6. Drumstick + bottle gourd	241.5	221.4	231.45	54.1	55.6	54.85	41.23	46.68	43.96
7. Drumstick + bitter gourd	282.9	274.6	278.75	50.7	51.4	51.05	19.31	23.14	21.24
8. Drumstick + ridge gourd	488.0	461.3	474.65	57.2	55.3	56.25	45.60	52.22	48.84
9. Drumstick + sponge gourd	475.1	470.8	472.95	53.6	49.3	51.45	36.12	40.15	38.12
10. Drumstick + snake gourd	353.4	329.3	341.35	61.8	66.3	64.05	26.88	32.60	29.68
CD at 5%	ns	ns		ns	ns		0.56	0.71	

G=ground spreading method, COD= climbed over drumstick

Table 2. Yield and economics of drumstick based multitier cropping system (pooled data)

Cropping system	Yield per plot (kg)	Total yield per ha (t)	Income of drumstick (Rs.)	Income of crops (Rs.)	Total income (Rs.)	Total expenditure (Rs.)
1. Drumstick	40.33	26.88	71725	-	71725	26585
2. Bottle gourd (G)	-	21.52	-	48829	48829	15882
3. Bitter gourd (G)	-	12.13	-	36400	36400	12364
4. Ridge gourd (G)	-	22.46	-	49412	49412	14229
5. Sponge gourd (G)	-	20.18	-	36336	36336	13247
6. Snake gourd (G)	-	14.26	-	39946	39946	13288
7. Drumstick + bottle gourd (COD)	38.23	54.77	63666	67405	131072	33586
8. Drumstick + bitter gourd (COD)	36.39	38.42	60650	42480	103130	33454
9. Drumstick + ridge gourd (COD)	36.98	57.21	61633	71632	133265	29781
10. Drumstick + sponge gourd (COD)	37.23	50.21	62000	45744	107744	31815
11. Drumstick + snake gourd (COD)	35.63	43.52	59333	43530	102864	30251
CD at 5%	ns	4.33				

G=ground spreading method, COD= climbed over drumstick

findings are supported by the results of Singh *et al.*, (2001) in sweet pepper indicating the yield reduction in the main crop when intercropped with non leguminous crops. Reddy *et al.*, (1993) also reported that the reduction in the yield of arecanut.

Economic feasibility

When the values of land equivalent ratio appears to be greater than one under intercropping system, this usually indicates the efficiency of this system over the sole cropping system (Vandermeer, 1989). In this study, the land equivalent ratio as an indicator of biological efficiency intercropping system was always greater than one (Table 3). Highest land equivalent ratio was obtained in the treatment combining drumstick with ridge gourd (2.39) i.e. 2.39 times area is required to get the same produce in their monoculture treatment. This multitier system approach not only reduced the space by 2.39 times but also neglected the use of trellis system of cultivation. The same treatment recorded the highest drumstick equivalent ration (28.65 t/ha). Willey (1979) reported that the practical significance of LER can only be fully assessed when related to the actual economic yield. However, Muonenke and Asiegbu (1997) concluded that the highest LER values did not always reflect highest monetary return to the farmer.

The sustainability of any intercropping system is influenced by the economic returns, which determine the commercial feasibility of different intercropping. The economics of various combination are given in Table 3. The maximum net profit of Rs 103484/- followed by Rs. 97486/- in drumstick + ridge gourd and drumstick + bottle gourd combination respectively. The most economically satisfactory intercropping system was obtained with drumstick + ridge gourd (3.47%), followed by drumstick + bottle gourd (2.98%). It is a compulsion of farmers of semi-arid region to grow crops in rainy season alone, for which investing in preparing trellis can be an economical. In the present study, the drumstick based multitier system reduced the cost input required for preparing trellis by 71 and 64 per cent comparing the bower and traditional system respectively as these two systems incurred Rs. 83000/- and Rs.67100/- respectively for preparing trellis (Table 4). Hence, it is concluded that the drumstick multitier cropping system, not only brings the drumstick grown areas under gourds and vice versa, but also facilitates to introduce more annual vegetables, favourable price during glut period, easy accessible for intercultivation enhance the input use efficiency. Similar observation are also noted by Patil *et al.*, (1992), Hegde *et al* (1993) and Nagwedat *et al.*, (1997) drumstick in coconut based vegetable cropping system. Hence, this system can be commercially exploited to the poor farming community.

Table 3. Profitability and compatibility of drumstick based multitier cropping system for semi-arid ecosystem (pooled data)

Cropping system	Net income (Rs)	C/B ratio	Drumstick reduction over pure crop (%)	Yield (%)	Supportive crop % Yield increase over pure crop	Drumstick equivalent yield (t/ha)	Land equivalent ratio
1. Drumstick	45140	1.69	-	-	-	-	1.00
2. Bottle gourd (G)	32947	2.07	-	-	-	19.80	1.00
3. Bitter gourd (G)	24036	1.94	-	-	-	14.56	1.00
4. Ridge gourd (G)	35183	2.47	-	-	-	19.76	1.00
5. Sponge gourd (G)	23089	1.74	-	-	-	14.53	1.00
6. Snake gourd (G)	26658	2.00	-	-	-	15.97	1.00
7. Drumstick + bottle gourd (COD)	97486	2.90	5.28	5.28	36.15	26.96	2.26
8. Drumstick + bitter gourd (COD)	69676	2.08	9.74	9.74	16.73	16.99	2.01
9. Drumstick + ridge gourd (COD)	103484	3.47	8.29	8.29	44.96	28.65	2.39
10. Drumstick + sponge gourd (COD)	75929	2.38	7.73	7.73	25.91	18.29	2.12
11. Drumstick + snake gourd (COD)	72613	2.40	11.71	11.71	38.70	22.16	2.21

G=ground spreading method, COD= climbed over drumstick

Table 4: Comparative performance of drumstick based multitier system over bower and traditional system

S. No.	Factors	System of cultivation		
		Bowar system	Traditional system	Drumstick based multitier system
1	Spacing for each poles	3.0 x 3.0 m	3.0 x 2.0 m	5.0 x 3.0 m
2	Cost for trellis formation	83600	67100	-
3	Cost of cultivation	16100	15800	29780
4	Yield (kg/ha)	30000	23700	57160
5	Total expenditure	99700	82900	29780
6	Total income	66000	52140	133265
7	Net Income	-33700	-30760	103485
8	Input reduction percent	71.0	64.0	-

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Effect of nitrogen on growth and nutrient status of bael (*Aegle marmelos* Correa) under sand culture

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Abstract

A pot experiment on Bael (*Aegle marmelos* Correa) seedlings was conducted during September 2005 to May 2006 in completely randomized design with three replications and nine treatments of each element at the Department of Horticulture, Govind Ballabh Pant University of Agriculture and Technology, Pantnagar, Udham Singh Nagar, Uttarakhand. Nine different treatments of nitrogen and phosphorus each were applied in the experiment. Results of the present investigation revealed that nitrogen and phosphorus deficiency resulted in reduced vegetative growth attributes of bael seedling. In nitrogen deficiency maximum height of seedling (13.67 cm), number of leaves (15.00), length of leaf (11.20 cm), breadth of leaf (3.23 cm) were found with T_3 (control) and total chlorophyll content (1.814 mg/g fresh weight) was found with T_6 . While minimum values were obtained with T_1 (without nitrogen). In the present results, the critical level of nitrogen was considered as 0.65 per cent on dry weight basis.

Key words: Bael, *Aegle marmelos*, leaf analysis, nitrogen deficiency, critical level.

Introduction

Bael (*Aegle marmelos* Correa) is one of the important minor fruit crops with high nutritive and medicinal value. It can thrive well in swampy, alkaline, stony soils. Bael tree flowers in May-June and fruits become ready by April-May. It requires substantial amount of nutrients for maximizing yield and improving quality. Hence, its nutrient requirements have to be carefully monitored through leaf analysis for high productivity and quality. No established standards are available for efficient nutrient programme and fixing of critical levels for the deficiency of nutrients. There are scanty reports in the literature about the development of deficiency symptoms and fixing critical levels of nutrients in fruit crops. Therefore, an investigation was carried out to develop nutrient critical levels in the leaves of bael seedlings which in addition showed the effect of nitrogen on growth attributes of bael seedlings.

Materials and methods

The experiment was conducted during September 2005 to May 2006 in the glass house at College of Agriculture of Govind Ballabh Pant University of Agriculture and Technology, Pantnagar, district Udham Singh Nagar (Uttarakhand), India. bael cv. PB 10 seedlings were initially raised in sand for a period of two months and then they were transplanted into plastic pots of size 25 cm diam-

eter, containing quartz sand. The sand was thoroughly washed with running tap water and later on immersed in solution prepared by dissolving 170 ml concentrated HCl and 10 g oxalic acid making up the volume of one litre in a volumetric flask with distilled water. The sand was kept in this solution for 48 hours. This treatment brought the pH of sand to near one. Later on washing was done with distilled water until the pH rose to 6.5. After transplanting, plants were kept in glass house. Complete nutrient solution as suggested by Hoagland and Arnon (1938) used to raise seedlings for the first 30 days, so that the planted material could establish itself well and acclimatize to the new anchoring medium. Stock solution of all the macro-elements and iron were prepared separately. A solution was also prepared containing all the macro-nutrients except Fe. Volume of working solutions was made up with double distilled water. Plants were supplied with 18 different of treatments including a control (complete nutrient solution). For nitrogen, treatments were N_1 (without nitrogen), N_2 $\frac{1}{4}x$ (25% of N), N_3 $\frac{1}{2}x$ (50% of N), N_4 $\frac{3}{4}x$ (75% of N), N_5 x (100% of N), N_6 $1.25x$ (125% of N), N_7 $1.5x$ (150% of N), N_8 $1.75x$ (175% of N), N_9 $2x$ (200% of N) and nine similar concentrations were given to the phosphorus. Composition of stock solution and method of making complete nutrient solution as suggested by Hewitt (1966).

The seedlings were transplanted in three plastic

pots each containing nine seedlings. Thus, there were 54 pots containing 486 seedlings. Each pot was supplied with 50 ml of nutrient solution every day split into two equal dose of 25 ml of nutrient solutions, throughout the experimental period till the last observations. Growth attributes i.e., height of seedling, number of leaves per seedling, leaf length and breadth, fresh and dry weight of leaves, leaf area, stem girth, length of primary root, chlorophyll a, chlorophyll b and total chlorophyll content were recorded at monthly intervals throughout the experimental period till the last observation. Leaf samples were collected at monthly intervals in the morning hours and then brought to the laboratory on the same day. After collection, leaf samples were washed in tap water and placed for drying of water. The samples were placed in muslin cloth bags and kept in oven at $60 \pm 2^\circ\text{C}$ for 48 hours to get constant dry weight. Grinding of the leaf samples was done with Rally Mixi to the powder form and analysed for nutrient content of leaves. For estimation of nitrogen digestion done by Kjeldhal method. For digestion of P, K, Ca, Mg, Cu, Mn, Fe and Zn used 9:4 of HNO_3 : HClO_4 solution. The data for the just noticeable deficiency symptoms of nutrients were noted and corresponding levels of nutrient content of each of the element was estimated as critical level of leaves.

Results and discussion

Data presented in Table 1 showed that the nitrogen deficient plants developed uniform chlorosis on the lower most leaves and progressed with the age of the seedling. They become pale green with yellowish tinge in the later stage. Different levels of nitrogen influenced all the growth characters significantly. Maximum height of seedling (13.76 cm), number of leaves (15.00), length of leaf (11.20 cm) and breadth of leaf (3.23 cm), fresh weight of leaves (1.10 g), dry weight of leaves (0.48 g), leaf area (85.00 cm^2), stem girth of seedling (1.39 cm) and length of primary root (19.96 cm) were observed with T_5 (100% of N) in contrast minimum values of height of seedling (9.90 cm), number of leaves (9.00), length of leaf (4.63 cm), breadth of leaf (1.96 cm), fresh weight of leaves (0.90 g), dry weight of leaves (0.29 g), leaf area (66.33 cm^2), stem girth (0.19 cm) and length of primary root (9.50 cm) were recorded with T_1 (without nitrogen) on the last date of observation. Maximum chlorophyll a (0.953 mg/g fresh weight), chlorophyll b (0.650 mg/g fresh weight) and total chlorophyll content (1.814 mg/g fresh weight) were recorded with T_5 as compared to minimum values of chlorophyll a (0.377 mg/g fresh weight), chlorophyll b (0.082 mg/g fresh weight) and total chlorophyll content (0.452 mg/g fresh weight) were recorded with T_1 treatment (without nitrogen) (Table 1) after 90 days of observation. Since nitrogen is a highly mobile element with the plant system, a stage reached where the plant felt a scarcity in nitrogen supply, nitrogen from older leaves translocated to the more active growing regions of the plant where nitrogen required for growth processes. Nitrogen

Table 1. Effect of variable nitrogen supply on growth attributes of Bael seedling.

Treatment	Height of seedling (cm)	Number of leaves	Length of leaf (cm)	Breadth of leaf (cm)	Fresh weight of leaves (g)	Dry weight of leaves (g)	Leaf area (cm^2)	Stem girth (cm)	Length of primary root (cm)	Chlorophyll a (mg/g of fresh weight of leaves)	Chlorophyll b (mg/g of fresh weight of leaves)	Total chlorophyll (mg/g fresh weight of leaves)
T_1	9.90	9.00	4.63	1.96	0.900	0.293	66.33	0.191	9.50	0.377	0.082	0.452
T_2	11.40	10.00	4.73	2.00	1.006	0.336	68.00	0.219	12.43	0.638	0.145	1.596
T_3	12.40	12.00	7.53	2.06	1.066	0.340	60.33	0.227	16.63	0.675	0.273	1.598
T_4	12.40	13.00	7.56	2.33	1.088	0.433	78.00	1.275	18.03	0.701	0.279	1.601
T_5	13.76	15.00	11.20	3.23	1.100	0.483	85.00	1.398	19.96	0.951	0.599	1.814
T_6	13.50	14.00	10.60	2.70	1.009	0.420	81.00	1.308	19.26	0.951	0.630	1.914
T_7	13.23	13.66	10.56	2.53	1.004	0.400	79.00	1.281	18.03	0.951	0.650	1.812
T_8	12.56	12.00	8.36	2.40	1.000	0.388	72.00	1.268	17.13	0.952	0.648	1.810
T_9	12.53	12.00	7.63	2.40	1.000	0.383	70.00	0.752	16.53	0.953	0.648	1.676
S.E.m ±	0.411	0.577	0.137	0.933	0.069	0.145	1.936	0.129	0.313	0.007	0.003	0.026
CD (P = 0.05)	1.231	1.650	0.409	0.396	0.206	0.136	3.781	0.383	0.931	0.021	0.011	0.077

Table 2. Effect of variable nitrogen supply on uptake of nutrients in Bael seedling.

Treatment	Nitrogen content of leaf (%)	Phosphorus content of leaf (%)	Potassium content of leaf (%)	Calcium content of leaf (%)	Magnesium content of leaf (%)	Copper content of leaf (ppm)	Iron content of leaf (ppm)	Manganese content of leaf (ppm)	Zinc content of leaf (ppm)
T ₁	0.24	0.31	0.98	1.28	0.70	41.11	42.33	82.00	76.66
T ₂	0.65	0.32	0.99	1.28	0.70	41.00	41.66	81.00	76.33
T ₃	0.65	0.32	1.00	1.28	0.70	41.66	41.66	81.00	76.00
T ₄	0.66	0.32	1.00	1.29	0.70	41.66	40.66	83.00	76.33
T ₅	1.36	0.33	1.04	1.30	0.71	41.33	40.33	85.00	76.66
T ₆	1.37	0.32	1.02	1.31	0.70	41.33	41.66	85.00	76.66
T ₇	1.40	0.32	1.01	1.30	0.70	41.00	41.00	85.00	76.33
T ₈	1.41	0.32	1.00	1.30	0.70	41.00	41.00	85.00	76.00
T ₉	1.45	0.32	1.01	1.30	0.70	41.00	40.66	85.00	76.00
S.E.m ±	0.010	0.008	0.012	0.011	0.007	0.430	1.110	1.36	0.683
CD (P=0.05)	0.035	NS	NS	NS	NS	NS	NS	NS	NS

influenced the growth of plant. As the levels of nitrogen reduced in the nutrient solution, there was corresponding decrease in growth attributes and up to a certain limit increase in the height of seedling. This is primarily due to the role of nitrogen in the growth process reported by Bhattacharya *et al.*, (1972).

The lowest nitrogen content of leaves (0.24%) with T₁, while maximum value of leaf nitrogen content (1.45%) was found with T₉ (maximum concentration of nitrogen) on the last date of sampling (Table 2). Motiramani (1968) reported that increasing nitrogen availability over a period of time increased the nitrogen uptake, thereby resulting into corresponding increase in plant nitrogen content. Other nutrients i.e. phosphorous, potassium, calcium, magnesium, copper, iron, manganese and zinc showed non-significant difference after 90 days of observation. The present results are, however, in line with the finding of Malik and Singh (1960). The critical level of leaf nitrogen was considered as 0.65 per cent on dry weight basis.

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Nutritional survey of aonla orchards in Sikar district of Rajasthan

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Abstract

Analysis of Aonla leaf samples showed that sulphur and manganese were found low in range whereas nitrogen, phosphorus, potassium, calcium and zinc were found low to medium in range. The magnesium, iron and copper were found in sufficient in range. The overall mean values of nitrogen, phosphorus, potassium, calcium, magnesium and sulphur were 0.842, 0.105, 0.384, 0.503, 0.387 and 0.0417 per cent, respectively whereas overall mean values of copper, manganese, zinc and iron were 9.34, 9.15, 10.04 and 120.66 mg kg⁻¹, respectively in aonla orchards.

Key words: Aonla, nutritional survey, copper, manganese, zinc, iron.

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 (Chandra and Chandra, 1997). 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 irrigation limited. Its cultivation is now becoming quite popular in irrigated arid regions of Rajasthan, owing to its high nutritive and medicinal value and having higher productivity even in the waste land. It is also a value added horticultural crop which has bright future prospects for export, particularly to European countries. Leaf analysis is a tool for determining the nutrient requirements of fruit plants and its nutrient status is an indication of growth and productivity. Very little information is available on nutritional status of aonla fruit crop under arid irrigated conditions of Rajasthan. Therefore, the present investigation was conducted during year 2007-2008 to survey the aonla orchard with a view to gaining information about their nutrient status.

Materials and methods

Studies were conducted in eight orchards of aonla selected at different locations in Sikar district of Rajasthan.

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These locations are given as under:-

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 Puran 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

In each orchard, five fruit trees with uniform size and vigour were selected. The methodology for leaf sampling as described by Anonymous, 1996 for aonla was followed. Composite leaf samples were taken in the month of July from the middle portion of 3-5 months old indeterminate shoots. The sample size was 50 leaves per sample and taken from all the identified five trees. These samples were washed with ordinary water containing dilute (0.2%) detergent solution for about two minutes and were then dipped into single distilled water, 0.1 N HCl and double distilled water. Samples were dried in an oven at 70°C for 24 hours and ground. The leaf samples were analysed for different nutrients. Nitrogen and phosphorus contents were determined calorimetrically by using Nessler's reagent and Vanadomolybdophosphoric acid yellow colour method (Jackson, 1967), respectively. Potassium content was estimated from digested aliquot of digestion II using flame photometer (Bhargava and Raghupati, 1993). Sulphur was estimated by turbidimetric method (Tabatabai and Bremner, 1970). Calcium and Magnesium were determined by Atomic spectro photometer (Bhargava and Raghupati, 1993) and micronutrients viz. Zn, Cu, Mn and Fe were determined from

Table 1. Soil fertility status of aonla orchards in Sikar district

Locations/ soil properties	Rashidpura	Rehnawa	Laxmangarh	Sargoth	Pachar I	Pachar II	Pachar VI	Palsana I
Available Nitrogen kg ha^{-1}	93.88	76.00	90.00	91.00	108.13	111.88	109.38	100.75
Available Phosphorous kg ha^{-1}	26.95	20.87	28.81	26.47	23.59	27.36	20.12	9.92
Available Potash kg ha^{-1}	120.38	112.75	117.75	115.50	130.38	145.75	143.00	123.88
Exchangeable Calcium cmol kg^{-1}	3.91	3.40	3.36	3.34	4.05	3.82	3.65	2.76
Exchangeable Magnesium cmol kg^{-1}	1.99	2.03	2.04	2.00	1.83	2.02	1.88	1.57
Available sulphur mg kg^{-1}	5.85	5.87	6.50	5.60	6.10	6.49	6.27	8.29
DTPA Extractable Fe mg kg^{-1}	2.41	4.13	3.21	3.72	4.56	5.25	6.40	5.93
DTPA Extractable Zn mg kg^{-1}	0.281	0.478	0.334	0.569	0.780	0.885	0.469	0.905
DTPA Extractable Cu mg kg^{-1}	0.149	0.153	0.154	0.139	0.265	0.300	0.412	0.341
DTPA Extractable Mn mg kg^{-1}	1.56	1.58	2.03	2.62	3.24	3.55	4.94	4.51

digested aliquot of digestion II using AAS (Lindsay and Norvell, 1978). Plant leaf nutrient parameters were tested in one way classification (Randomized Complete Block Design) with three replications.

Results and discussion

Data on the determination of macro and micronutrients status of aonla orchards in Sikar district are presented in Table 1. The range and mean values of N, P, K, Ca, Mg, S, Cu, Zn, Mn and Fe contents of the aonla leaves showed that there were wide variations for these nutrients amongst the different orchards studied.

The nitrogen content of aonla leaves ranged between 0.458 to 1.568 per cent with mean value 0.842 per cent. The minimum nitrogen content (0.458%) was obtained at the orchard of Rehnawa, while maximum value (1.568%) was obtained at the orchard of Pachar-II. Analysis of aonla leaf samples revealed that 88 per cent leaf samples were found low and 12 per cent leaf samples were found sufficient in nitrogen content. The low concentrations of nitrogen could be attributed to low nitrogen status of soils (88.63 to 106.13 kg ha^{-1}), poor organic matter (0.098 to 0.136 per cent), high pH (8.21 to 8.67) and inadequate application of nitrogen. The results of present investigations are in accordance with those reported by Bhatnagar *et al.*, (2001), Bhatnagar and Chandra (2006 and 2007) and Rohitash

(2007).

The content of phosphorus in aonla leaves ranged from 0.141 to 0.184 per cent with mean value 0.165 per cent. The maximum phosphorus content (0.184%) was obtained at the orchard of Laxmangarh, while minimum content (0.41%) was obtained at the orchard of Palsana-I. The evaluation of aonla leaf samples showed that 50 per cent leaf samples were found low and remaining 50 per cent leaf samples were found sufficient in phosphorus content. The variation in phosphorus content of aonla fruit crop is in accordance to the available phosphorus status of the orchard soils (21.49 to 25.56 kg ha^{-1}) and proper uptake and utilisation by the plant tissues. The present findings are in conformity with the findings of Reddy *et al.* (1991), Bhatnagar and Chandra (2006 and 2007).

The potassium content of aonla leaves ranged from 0.210 to 0.580 per cent with mean value 0.384 per cent. The maximum potassium content (0.580%) was obtained at the orchard of Pachar-II, while minimum content (0.210%) was obtained at the orchard of Rehnawa. Analysis of aonla leaf samples revealed that 37 per cent leaf samples were found low and 63 per cent leaf samples were found sufficient in potassium content. The fair potassium content in aonla leaves might be due to medium potassium status of orchard soils (113.38 to 139.00 kg ha^{-1}). The present find-

Table 2. Macro and Micronutrient contents (oven dry basis) in leaf samples of Aonla collected from orchards in Sikar District

Location	Macro nutrients (%)					Micro nutrients (mg kg ⁻¹)				
	N	P	K	Ca	Mg	S	Cu	Mn	Zn	Fe
Rashidpura	0.742	0.181	0.330	0.630	0.398	0.0376	7.44	5.85	6.24	83.71
Rehawa	0.458	0.157	0.210	0.430	0.426	0.0396	8.49	9.67	9.53	107.57
Laxmangarh	0.500	0.184	0.260	0.390	0.466	0.0408	9.20	6.21	8.40	92.56
Sargoth	0.576	0.180	0.300	0.350	0.472	0.0356	5.72	6.69	11.18	101.64
Pachar - I	0.968	0.158	0.520	0.810	0.32	0.0425	10.01	10.11	11.54	125.53
Pachar - II	1.568	0.167	0.580	0.590	0.364	0.0459	11.10	10.58	11.94	145.28
Pachar - VI	1.054	0.150	0.480	0.530	0.358	0.0459	11.48	12.46	8.84	158.32
Palsana - I	0.872	0.141	0.390	0.290	0.294	0.0459	11.25	11.60	12.62	150.69
Mean	0.842	0.165	0.384	0.503	0.387	0.0417	9.34	9.15	10.04	120.66
Range	0.458 to 1.568	0.141 to 0.184	0.210 to 0.580	0.290 to 0.810	0.294 to 0.472	0.0356 to 0.0459	5.72 to 11.48	5.85 to 12.46	6.24 to 12.62	83.71 to 158.32
SEm ±	0.083	0.003	0.035	0.027	0.019	0.0011	0.50	0.54	0.55	5.95
CD (5%)	0.238	0.009	0.101	0.078	0.056	0.0031	1.45	1.55	1.60	17.15
Low (%)	88	50	37	50	-	100	-	100	13	-
Sufficient (%)	12	50	63	50	100	-	100	-	37	100
High (%)	-	-	-	-	-	-	-	-	-	-

ings are in conformity with the findings of Reddy *et al.* (1991), Bhatnagar *et al.*, (2001) and Bhatnagar and Chandra (2006 and 2007).

The calcium content of aonla leaves varied between 0.290 to 0.810 per cent with mean value 0.503 per cent. The maximum calcium content (0.810%) was obtained at the orchard of Pachar-I, while minimum content (0.290%) was obtained at the orchard of Palsana-I. Analysis of aonla leaf samples revealed that 50 per cent leaf samples were found low and remaining 50 per cent leaf samples were found sufficient in calcium content. Sufficient calcium content in leaves may be due to presence of high amount of calcium (2.59-4.29 cmol kg⁻¹) in orchard soils, whereas low calcium content in some of the orchards could be due to low irrigation frequency, because the movement of calcium in plant is facilitated by higher amount of available water in the soil. These results of present study are in confirmation with those reported by Rohitash (2007) in aonla.

The magnesium content of aonla leaves varied between 0.294 to 0.472 percent with mean value 0.387 per cent. The maximum magnesium content (0.472%) was obtained at the orchard of Sargoth, while minimum content (0.294%) was obtained at the orchard of Palsana-I. Analysis of aonla leaf samples revealed that all the leaf samples

were found sufficient in magnesium content. The results are in conformity with the finding of Kumawat (2005) in ber orchards in Jaipur district and Rohitash (2007) in aonla.

The sulphur content of aonla leaves varied between 0.0356 to 0.0459 percent with mean value 0.0417 per cent. The maximum sulphur content (0.0459%) was obtained at the orchard of Palsana-I, Pachar-II and Pachar-IV while minimum content (0.0356%) was obtained at the orchard of Sargoth. The data shows that all the leaf samples were found low in sulphur content. The low content of sulphur in leaves of various fruit trees could be due to low sulphur status of soil (5.99 to 6.91 mg kg⁻¹) of the studied orchards. The results of present study are in conformity with the findings of Gathala *et al.* (2004) in pomegranate and Rajkumar (2004) in ber and pomegranate.

The copper content of aonla leaves varied between 5.72 to 11.48 mg kg⁻¹ with mean value 9.34 mg kg⁻¹. The maximum copper content (11.48 mg kg⁻¹) was obtained at the orchard of Pachar-VI, while minimum content (5.72 mg kg⁻¹) was obtained at the orchard of Sargoth. Analysis of aonla leaf samples revealed that all the leaf samples were found sufficient in copper content. The variations in copper content of leaves could be explained in accordance to the availability of nutrients in soil, variation in pH and or-

ganic carbon status. Similar findings were also reported by Sharma and Mahajan (1990), Bhatnagar *et al.*, (2001), Gathala *et al.* (2004), Bhatnagar and Chandra (2006 and 2007) and Rohitash (2007).

The manganese content of aonla leaves varied between 5.85 to 12.46 mg kg⁻¹ with mean value 9.15 mg kg⁻¹. The maximum manganese content (12.46 mg kg⁻¹) was obtained at the orchard of Pachar-VI, while minimum content (5.85 mg kg⁻¹) was obtained at the orchard of Rashidpura. The analysis of aonla leaf samples showed that all the leaf samples were found low in manganese content. The location wise variations in manganese content of all fruit crops studied could be explained in accordance to the availability of nutrients in soil, variation in pH and organic carbon status. Similar findings were reported by Bhatnagar *et al.*, (2001), Bhatnagar and Chandra (2006 and 2007) and Rohitash (2007).

The zinc content of aonla leaves varied between 6.24 to 12.62 mg kg⁻¹ with mean value 10.04 mg kg⁻¹. The maximum zinc content (12.62 mg kg⁻¹) was obtained at the orchard of Palsana-I, while minimum content (6.24 mg kg⁻¹) was obtained at the orchard of Rashidpura. Analysis of aonla leaf samples revealed that 13 per cent leaf samples were found low and 87 per cent leaf samples were found sufficient in zinc content. The location wise variations in zinc content of all the taken fruit crops studied could be explained in accordance to the availability of nutrients in soil, variation in pH and organic carbon status. The result of study is in accordance to those reported by Bhatnagar and Chandra (2006) and Rohitash (2007).

The iron content of aonla leaves varied between 83.71 to 158.32 mg kg⁻¹ with mean value 120.66 mg kg⁻¹. The maximum iron content (158.32 mg kg⁻¹) was obtained in the orchard Pachar-VI, while minimum content (83.71 mg kg⁻¹) was obtained in the orchard at Rashidpura. Analysis of aonla leaf samples revealed that all the leaf samples were found sufficient in iron content. The location wise variations in iron content of all the taken fruit crops studied could be explained in accordance to the availability of nutrients in soil, variation in pH and organic carbon status. Similar results were reported by Bhatnagar and Chandra (2006 and 2007) and Rohitash (2007).

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Genotyping of some elite date palm germplasm using RAPD marker

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Abstract

In India wild groves of seedling date palms are found on the coastal belt from Anjar to Mandvi in Kachchh district of Gujarat and these groves are believed to be 200 years old. A great diversity is existing in morphological characteristics of indigenous date palms of Kachchh, hence molecular characterization of this precious genetic resource is significant. Improvement of date palm through conventional breeding methods is very difficult due to its long life cycle, strongly heterogeneous nature and impossibility to determine the sex at early stages of development. Marker assisted selection based on molecular characterization can overcome these hurdles. The study was done at Date palm Research Station, Mundra and C.P. College of Agriculture, Sardarkrushinagar, Gujarat. RAPD fingerprints were performed on DNA extracted from 10 elite date palm germplasm. The analysis had two objectives - to detect polymorphism within cultivars of date palm and to investigate the genetic diversity of the indigenous date palm germplasm of Kachchh. Out of eight random primers screened, two primers OPD 16 and OPD 20 produced comparatively good polymorphic bands. The efficiency of RAPD as a simple molecular marker for cultivar identification and genetic relationships among different date palm germplasm are discussed.

Keywords: Date palm (*Phoenix dactylifera* L.), RAPD polymorphism, genetic diversity.

Abbreviations: CTAB, Hexadecyltrimethylammonium bromide; EDTA, Disodium ethylene diamine tetraacetate; PCR, Polymerase chain reaction.

Introduction

Date palm (*Phoenix dactylifera* L.), is one of the important cultivated crops in arid regions of the world. The date belt stretches from the Indus valley in the east to Atlantic Ocean in the West. At present in India, commercial cultivation of this crop is restricted to Kachchh district of Gujarat. The wild date groves found in this area are rich in genetic diversity, which is due to the dioecious nature of date palm and cross-pollination. Besides its socio-economic importance the date groves contribute a lot to the stability of arid environment that have been tainted due to tremendous industrialization in the area. RAPD (Random Amplified Polymorphic DNA) technique (Williams *et al.*, 1990) has been widely used as a method to identify genetic polymorphism in several crops, which in turn helps to assess genetic diversity and to take measures to improve the existing varieties or to protect the genetic resource for further crop improvement researches. In the present study RAPD molecular marker was used to examine the genetic polymorphism in the available elite date palm germplasm which are suitable to agro-climatic conditions of Kachchh.

Materials and methods

Plant material

A set of 10 date palm germplasm (Table 1) has been used for the study of which four are exotic varieties (label 1-4) and the remaining six are local germplasm (label 5-10). The plant material consists of the young leaves collected from Date palm Research station, S. D. Agricultural University, Mundra, Kachchh.

Table 1. List of date palm germplasm

Germplasm	Label	Exotic/Indigenous
Barhee	1	Exotic
Halawy	2	Exotic
Khadrawy	3	Exotic
Khalas	4	Exotic
MDP-02	5	Indigenous
MDP-03	6	Indigenous
MDP-05	7	Indigenous
MDP-06	8	Indigenous
MDP-09	9	Indigenous
MDP-10	10	Indigenous

MDP = Mundra Date Palm

DNA extraction

DNA extraction performed in Biotechnology Laboratory, C. P. College of Agriculture, Sardarkrushinagar, Gujarat Total cellular DNA of 10 germplasm was extracted from 1 g leaflets according to Doyle and Doyle (1990) with some modifications. The leaves were first ground into fine powder with liquid nitrogen. DNA was extracted in 10 ml extraction buffer (3% CTAB, 1 M Tris pH 8, 0.2 M EDTA and 1.4 M NaCl) for one hour at 65°C. After complete extraction of DNA, it was purified by treating with RNase (5 mg/ml) and phenol : chloroform : isoamyl alcohol in 25 : 24 : 1 ratio (v/v). The purified DNA pellet was washed in 70 per cent ethanol and dissolved in TE buffer (10 mM Tris HCl, pH 8, 0.1 mM EDTA).

Primers and RAPD assay

A total of eight primers were tested to amplify the isolated DNA. These primers are listed in Table 2. For PCR amplifications, a 20 µl reaction mixture was used which contained 10x Taq DNA polymerase buffer, 1.5 mM MgCl₂, 0.25 mM each of dATP, dCTP, dGTP and dTTP, 30 ng template DNA, 50 pm oligonucleotide primer and 1 unit of Taq DNA polymerase.

Table 2. List of RAPD primers with sequence

Primer	Nucleotide sequence (5' to 3')
OPA1	CAG GCC CTT C
OPA2	TGCCGAGCTG
OPA9	GGG TAACGCC
OPC2	GTGAGGCGTC
OPD16	AGG GCG TAAG
OPD20	ACC CGG TCA C
OPF13	GGCTGCAGAA
OPF20	GGTCTAGAGG

Amplifications were performed in a thermal cycler (Eppendorf Master Cycler®) and the apparatus was programmed to execute the following conditions: an initial denaturation step of 4 min at 93°C, followed by 40 cycles composed of 1 min at 93°C, 1 min at the annealing temperature (37°C), and 2 min at 72°C. A final extension of 72°C for 8 minutes was included. The amplified DNA fragments were visualized along with a DNA marker on 1.8% agarose gel with 1X TAE buffer and detected by staining with ethidium bromide. Gels were photographed on polaroid films under UV light.

Data analysis

For each DNA sample, RAPD bands were transformed into a binary matrix where the presence of polymorphic DNA band at particular position on gels is scored 1, while a 0 denotes the absence. The index of similarity between each two cultivars was calculated using the formula:

$B_{ab} = 2 N_{ab} / (N_a + N_b)$, where N_{ab} is the number of common fragments observed in individuals a and b, and N_a and N_b are the total number of fragments observed in individuals a and b respectively (Lynch, 1990). The genetic similarity among the different germplasm was calculated with the available banding patterns.

Results and discussion

A total of eight primers of arbitrary nucleotide sequence primers were screened for their ability to generate consistently amplified banding patterns and to assess polymorphism in the tested date palm cultivars. Among these primers, only two (OPD - 16 and OPD - 20) revealed polymorphic and unambiguously scorable bands. A set of four RAPD primers (Table 3) including OPD - 16 produced unique polymorphic bands useful to identify different germplasm. While smear or no amplified products were observed with the other primers. Details of RAPD profiles made by each primer are listed in Table 4. Examples of polymorphism are shown in Figure 1.

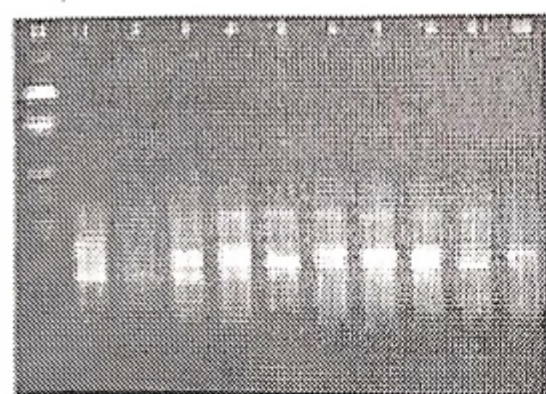
Table 3. Date palm cultivars identified using different RAPD primers.

Primer	No. of polymorphic bands	Varieties discriminated
OPA-2	1	MDP-02
OPD-16	3	MDP-09 and MDP-10
OPF-13	1	MDP-10
OPF-20	1	MDP-06
Total	6	Four

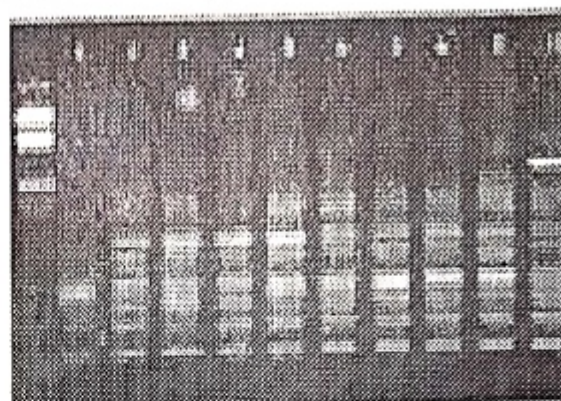
Table 4. Details of RAPD polymorphism detected using different primers.

Primer	Amplified bands	Total bands	No. of Polymorphic % of polymorphism
OPA-1	8	1	12.5
OPA-2	7	1	14.3
OPA-9	8	0	0
OPC-2	Smear	—	—
OPD-16	10	3	30.0
OPD-20	8	2	25.0
OPF-13	7	1	14.3
OPF-20	10	1	10.0

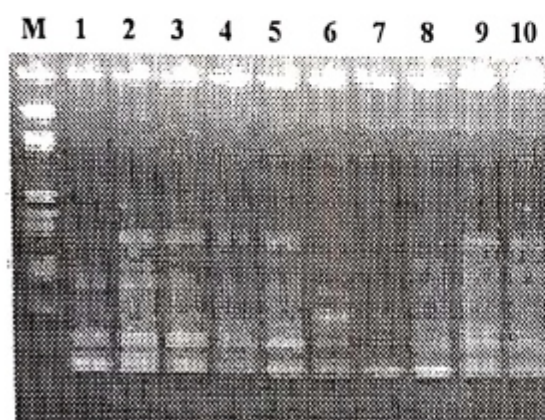
The matrix compiled using the data available from RAPD profile of primer OPD-16 showed a genetic similarity of 0.842 to 0.947 (Table 5) among the different local germplasm. Thus it may be assumed that these ecotypes emerged in Kachchh regions are characterized by a high degree of genetic similarity at the DNA level. The least similarity (0.842) was observed between MDP-10 and MDP-03 indicating that among the local germplasm these two



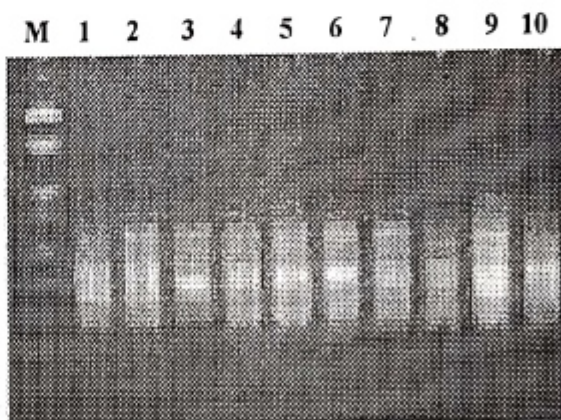
OPA-2



OPD-16



OPD-20



OPF-13

Figure 1: RAPD polymorphism in 10 samples of date palm revealed with the four random primers.

M: Standard molecular size marker (2 kb); lanes 1-10 accessions described in materials and methods and Table 1.

have the greatest genetic difference, but the data is not sufficient to draw any conclusion regarding the genetic similarity between exotic and local date palm germplasms.

Table 5. Similarity index based on RAPD profile using primer OPD-16.

Cultivars	MDP-03	MDP-05	MDP-09
MDP05	0.947		
MDP09	0.947	0.947	
MDP 10	0.842	0.947	0.900

There may be reason to view with caution systematic conclusions based on RAPD analysis alone. As reported by Williams *et al.* (1993), the small alterations in PCR parameters or quality of target DNA can alter RAPD patterns. Further standardization of DNA extraction procedure, PCR parameters and gel documentation process may overcome the appearance of unclear bands and smear formation. Use of more number of primers will be useful for the complete varietal identification of all the tested date palm cultivars. This study can be extended for accurate identification of date palm varieties cultivated and wild relatives of *Phoenix* germplasm collection, which will accelerate the crop improvement research using marker assisted selection (MAS).

Collection, conservation and documentation variable genetic resource of the date palm available in Kachchh is significant for crop improvement work. The results from

this study suggest that the use of RAPD markers to detect polymorphism is useful for identification of date palm germplasm.

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Line x tester analysis for combining ability in okra [*Abelmoschus esculentus* (L.) Moench.]

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Abstract

Combining ability effects were estimated for different characters in a line x tester crossing programme comprising 45 hybrids produced by crossing of 15 lines and 3 testers. Parents and hybrids differed significantly for gca and sca effects, respectively. High average degree of dominance revealed predominance of non-additive gene effects for all the traits. The parents Swati-10, Heritage green, Punjab Padmini, Ankur-40, VRO-6 and Arka Anamika were good general combiner for majority of characters in various environments. Therefore, these lines can be used for hybridization for producing promising recombinants. This indicates that parent showing high gca for fruit yield per plant might be due to high gca for fruit weight, fruit length, fruit girth and number of fruits per plant. High sca effects were reported for cross combinations, BO-3 x Arka Anamika in E₁, Swati-10 x Parbhani Kranti and heritage Green x Arka Abhay in E₂ and BO-3 x Parbhani Kranti in E₃ environment.

Key words: Combining stability, Line, Tester, Gca effects, Sca effects, Gene effects, Non-additive, Dominance, parents and Hybrids.

Introduction

Okra [*Abelmoschus esculentus* (L.) Moench], commonly known as bhindi has a prominent position among vegetable due to its wide adaptability, year round cultivation, export potential and high nutritive value. In any sound breeding programme, the proper choice of parents based on their combining ability is a prerequisite. As such studies intended to determine the combining ability not only for necessary information regarding the choice of parents but also the simultaneous nature and magnitude of desirable traits. Accordingly the present investigation was undertaken to have an idea of the nature of the gene action for green fruit yield and other important attributes in okra. Line x tester analysis is a useful tool for preliminary evaluation of genetic stock for use as combiners, which may be used to build up a population with favourable fixable and genes for effective yield improvement.

Materials and methods

The experimental material comprised of 15 female parents (lines) viz. Heritage green, Go-2, Bo-2, Punjab Padmini, Swati-10, Swati-25, Ankur-40, Pusa Sawani, VRO-5, VRO-6, Varsha Uphar, and 3 Pollen parents (testers) viz. Parbhani kranti, Arka abhay and Arka anamika were selected on the basis of per se performance, adoption and geographical diversity. They were crossed in the line x tester design thus 45 hybrids were produced. The 45 F₂s and their parents were grown in randomized block design with three replications at Horticulture Farm, Rajasthan College

of Agriculture, MPUAT, Udaipur, (24°-35°N and 73°-42°E) at an elevation of 582.17 above mean sea level. The climate of Udaipur is semi-arid sub-tropical, characterized by hot summers and cold winters. The hottest months are April, May and June (Maximum temperature 43-44° C), whereas during December and January, the minimum temperature often goes below 5 °C. The average annual rainfall is 750 mm, 70-80 % of which is received through the north-west monsoon during July-September. The soil was sandy loam in texture consisting 62.3 % sand, 21.6 % silt and 16.5 % clay having soil pH 7.8, electrical conductivity 0.46 dS/m, organic carbon 0.54 %, available N 73 mg/kg, Olsen P 14.3 mg/kg and available K 70.2 mg/kg of soil. The experiment was conducted in three environments viz., summer 2005 (E₁), rainy 2005 (E₂) and summer 2006 (E₃). The seeds were sown in single row of 3.6 m length keeping 45 cm distance between row and 30 cm within row. The observations were recorded for yield and yield contributing traits viz. plant height (cm), number of branches per plant, height of first effective fruiting node (cm), days to 50 per cent flowering, number of fruits per plant, fruit length (cm), fruit girth (cm), days to harvest, fruit weight (g) and yield per plant (g). Combining ability analysis was computed according to the model given by Kempthorne (1957).

Results and discussion

The analysis of variance revealed highly significant difference among all the traits which indicated the presence of considerable amount of genetic variability (Table 1). The mean square due to crosses, lines and testers

Table 1. Summation of combining ability in various environments

Source	Plant height	Number of branches Per plant	Height of first effective fruiting node	Days to 50% flowering	Number of fruits per plant	Fruit length	Fruit girth	Days to harvesting	Fruit weight	Yield per plant
E-1 Environment										
Σ^2 GCA T	24.168**	-0.005	0.15631	-0.07713	4.115**	0.15479*	0.037526**	-0.03922	0.19582**	1411.6**
Σ^2 GCA L	1097.1**	0.65395**	4.2829	23.463**	28.442**	3.8868**	0.39772**	21.832**	18.469**	14049**
Σ^2 SCA	2985.3**	5.4193**	99.878**	111.55**	200.76**	32.276**	2.0932**	116.38**	90.398**	79631**
E-2 Environment										
Σ^2 GCA T	32.766**	0.061853**	0.10106	0.1019	0.42326*	0.020297	0.02916**	0.16089**	3.0436**	-60.93
Σ^2 GCA L	886.55**	1.4979**	8.0278*	70.209*	16.064**	2.8888**	0.42754**	70.797**	31.065**	5841.6**
Σ^2 SCA	1751.1*	10.966**	74.471**	258.22**	87.393**	26.417**	1.6944**	272.59**	157.75**	33306**
E-3 Environment										
Σ^2 GCA T	32.738**	0.093974**	0.5839**	0.22308*	1.9553**	0.03022	0.038759**	0.12262	0.77404**	583.9**
Σ^2 GCA L	939.01**	1.1056**	9.294**	11.047**	17.656**	3.7167**	0.4354**	10.983**	7.6846**	9325**
Σ^2 SCA	2348.7**	10.69**	88.699**	55.557**	120.64**	31.122**	2.0524**	60.909**	53.7**	50453**

Table 2. Best parents identified on the basis of per se performance and GCA effects for various traits in different environment in okra

S. No.	Character	Per Se	GCA	Common
1.	Plant Height	E ₁ L ₁ , L ₁₃ , L ₉ , L ₄ E ₂ L ₁₄ , L ₁ , T ₁ , L ₄ , T ₂ E ₃ L ₁ , L ₁₃ , T ₁ , L ₁₁ , L ₉	L ₄ , L ₁₁ , L ₁₃ , L ₉ , L ₁ L ₄ , L ₁₁ , L ₁ , L ₁₃ , L ₉ L ₄ , L ₁₁ , L ₉ , L ₁ , L ₁₃	All L ₁ , L ₄ L ₁ , L ₁₁ , L ₁₃ , L ₉ , L ₄
2.	Number of branches per plant	E ₁ L ₁₄ , L ₉ , L ₁₃ , L ₇ , T ₃ E ₂ L ₉ , L ₁₄ , L ₁₃ , L ₄ , T ₃ E ₃ L ₄ , L ₁₄ , L ₁₃ , T ₁ , T ₃	L ₆ , L ₇ , L ₁ , L ₈ , L ₁₁ L ₉ , L ₆ , T ₃ , L ₁₃ , L ₁ L ₆ , L ₉ , L ₁₃ , T ₃ , T ₁₁	L ₇ L ₉ , T ₃ L ₁₃ , T ₃
3.	Height of first effective fruiting node	E ₁ L ₄ , L ₁₅ , L ₁ , L ₁₁ , L ₅ , L ₆ E ₂ L ₈ , L ₅ , L ₃ , L ₂ , L ₁ E ₃ L ₂ , L ₅ , L ₁₂ , L ₈ , T ₂	L ₄ , L ₇ , L ₁₂ , L ₁ , L ₁₀ L ₁₅ , L ₄ , L ₅ , L ₁ , T ₃ L ₁ , L ₆ , L ₂ , L ₇ , T ₂	L ₄ , L ₁ L ₅ , L ₁ L ₂ , T ₂
4.	Days to 50% flowering	E ₁ L ₁₁ , T ₃ , T ₂ , L ₁₂ , L ₈ E ₂ L ₄ , L ₁₁ , L ₁₃ , T ₃ , L ₆ , L ₈ E ₃ L ₄ , L ₅ , L ₁₁ , T ₁ , L ₉	L ₉ , L ₆ , L ₁₁ , L ₁ , L ₇ L ₁₁ , L ₁₃ , L ₁ , L ₉ , T ₁ L ₁₃ , L ₉ , L ₁₁ , T ₁ , L ₄	L ₁₁ L ₁₁ , L ₁₃ L ₄ , L ₁₁ , T ₁
5.	Number of fruits per plant	E ₁ T ₂ , L ₉ , L ₁₅ , L ₄ , L ₈ E ₂ L ₁₁ , T ₃ , L ₄ , L ₉ , L ₃ E ₃ L ₁ , L ₃ , T ₂ , T ₃ , L ₉	L ₁₁ , L ₄ , L ₁₃ , L ₁ , T ₃ L ₁₁ , L ₄ , L ₉ , L ₁₃ , T ₃ L ₁₃ , L ₁₁ , L ₉ , L ₁ , T ₃	L ₄ L ₁₁ , L ₄ , L ₉ , T ₃ L ₁ , L ₉ , T ₃
6.	Fruit Length	E ₁ L ₁ , L ₆ , L ₁₅ , L ₁ , L ₁₁ , L ₆ , L ₁ , L ₁₁ , L ₁₅ , L ₁₂ E ₂ L ₆ , L ₁ , L ₁₅ , L ₁₂ E ₃ L ₁ , L ₆ , L ₁₅ , L ₁₁ , L ₁₂	L ₁ , L ₆ , L ₈ , L ₁₀ , L ₄ L ₆ , L ₄ , L ₁₂ , L ₁₀ , L ₁ L ₄ , L ₁ , L ₁₂ , L ₆ , L ₁₀	L ₆ L ₆ , L ₁₂ L ₁ , L ₆ , L ₁₂
7.	Fruit girth	E ₁ T ₂ , L ₃ , L ₁₄ , L ₁₅ , L ₁₀ E ₂ T ₂ , L ₃ , L ₁₄ , L ₁₅ , L ₁₀ , T ₂ , L ₃ E ₃ L ₁₄ , L ₁₅ , L ₁₀	L ₃ , L ₁₄ , T ₂ , L ₁₅ , L ₁₀ L ₁₄ , L ₃ , L ₁₅ , L ₈ , T ₂ L ₁₄ , L ₁₅ , L ₁₅ , T ₂ , L ₈	L ₃ , L ₁₄ , L ₁₅ , L ₁₀ , T ₂ L ₃ , L ₁₄ , L ₁₅ , T ₂ L ₃ , L ₁₄ , L ₁₅ , T ₂
8.	Days to harvesting	E ₁ L ₁₁ , T ₃ , L ₁₂ , T ₂ , L ₃ , L ₈ E ₂ L ₄ , L ₁₁ , L ₁₃ , T ₃ , L ₈ E ₃ L ₄ , L ₅ , T ₁ , L ₁₁ , L ₉	L ₉ , L ₆ , L ₇ , L ₁₁ , L ₁ L ₁₁ , L ₁₃ , L ₁ , L ₉ , T ₁ L ₁₃ , L ₉ , L ₁₅ , L ₁ , L ₁₁	L ₁₁ L ₁₁ , L ₁₃ L ₁₁ , L ₉
9.	Fruit weigh	E ₁ L ₂ , L ₁₁ , T ₃ , L ₉ , L ₄ E ₂ L ₂ , L ₄ , T ₃ , L ₆ , L ₉ E ₃ L ₄ , L ₇ , L ₉ , L ₂ , T ₃	L ₉ , L ₁₁ , L ₁ , L ₄ , L ₁₃ L ₁₁ , L ₉ , L ₄ , L ₄ , L ₁ , T ₃ L ₉ , L ₁₃ , L ₄ , L ₁ , T ₃	L ₁ , L ₄ L ₄ , T ₃ L ₄ , L ₉ , T ₃
10.	Yield per plant	E ₁ L ₉ , L ₄ , L ₂ , L ₁₁ , T ₃ E ₂ L ₉ , T ₃ , L ₄ , L ₁₃ , L ₁₁ E ₃ T ₁ , T ₂ , T ₃ , L ₄ , L ₁₂	L ₁₁ , L ₁₃ , L ₉ , L ₁ , L ₄ L ₄ , L ₁₁ , L ₉ , L ₁ , L ₃ L ₁₂ , L ₁₄ , L ₁ , L ₇ , L ₄	L ₁₁ , L ₄ L ₁₁ , L ₄ , L ₉ L ₄

Table 3. Best hybrids identified on the basis of per se performance and SCA effects for various traits in different environments in okra

S. No.	Character		Per Se	SCA	Common
1.	Plant Height	E ₁	L ₁₁ x T ₃ , L ₄ x T ₃ , L ₄ x T ₂ , L ₁₁ x T ₃ , L ₁ x T ₃	L ₄ x T ₁ , L ₅ x T ₂ , L ₁ x T ₃ , L ₁₂ x T ₃ , L ₁₁ x T ₃	L ₁₁ x T ₃ , L ₁ x T ₃
		E ₂	L ₁₁ x T ₃ , L ₄ x T ₃ , L ₄ x T ₂ , L ₁ x T ₃ , L ₄ x T ₂	L ₄ x T ₃ , L ₉ x T ₂ , L ₅ x T ₁ , L ₄ x T ₁ , L ₇ x T ₃	L ₄ x T ₃ , L ₉ x T ₄
		E ₃	L ₁₁ x T ₃ , L ₄ x T ₃ , L ₉ x T ₃ , L ₁₁ x T ₂ , L ₁ x T ₃	L ₈ x T ₁ , L ₉ x T ₃ , L ₁₅ x T ₂ , L ₁₄ x T ₂ , L ₁ x T ₃	L ₉ x T ₃ , L ₁ x T ₃
2.	Number of branches per plant	E ₁	L ₄ x T ₃ , L ₉ x T ₁ , L ₆ x T ₂ , L ₇ x T ₂ , L ₅ x T ₂	L ₄ x T ₃ , L ₉ x T ₂ , L ₁₀ x T ₃ , L ₅ x T ₂ , L ₁₄ x T ₁	L ₄ x T ₃ , L ₉ x T ₁ , L ₅ x T ₂
		E ₂	L ₉ x T ₁ , L ₉ x T ₃ , L ₆ x T ₂ , L ₁₃ x T ₃ , L ₁₁ x T ₂	L ₁₀ x T ₃ , L ₁₁ x T ₂ , L ₅ x T ₂ , L ₉ x T ₁ , L ₆ x T ₂	L ₉ x T ₁ , L ₆ x T ₂ , L ₁₁ x T ₂
		E ₃	L ₁₁ x T ₃ , L ₆ x T ₃ , L ₁₃ x T ₂ , L ₁ x T ₁ , L ₃ x T ₃	L ₁₅ x T ₂ , L ₅ x T ₂ , L ₇ x T ₁ , L ₁ x T ₁ , L ₁₃ x T ₃	L ₁₅ x T ₂ , L ₁ x T ₁
3.	Height of first effective fruiting node	E ₁	L ₈ x T ₂ , L ₇ x T ₁ , L ₄ x T ₂ , L ₃ x T ₃ , L ₁₃ x T ₁	L ₈ x T ₂ , L ₃ x T ₃ , L ₁₄ x T ₃ , L ₁₃ x T ₁ , L ₇ x T ₁	L ₈ x T ₂ , L ₇ x T ₁ , L ₃ x T ₃ , L ₁₃ x T ₁
		E ₂	L ₄ x T ₁ , L ₁₁ x T ₂ , L ₁₅ x T ₂ , L ₃ x T ₃ , L ₆ x T ₃	L ₁₁ x T ₂ , L ₄ x T ₃ , L ₈ x T ₂ , L ₆ x T ₃ , L ₁ x T ₁	L ₁₁ x T ₂ , L ₆ x T ₃
		E ₃	L ₇ x T ₃ , L ₁ x T ₂ , L ₉ x T ₁ , L ₁₂ x T ₁ , L ₁₃ x T ₂	L ₁₂ x T ₁ , L ₃ x T ₃ , L ₉ x T ₁ , L ₇ x T ₃ , L ₈ x T ₂	L ₇ x T ₃ , L ₉ x T ₁ , L ₁₂ x T ₁
4.	Days to 50% flowering	E ₁	L ₁₁ x T ₃ , L ₉ x T ₁ , L ₁₃ x T ₁ , L ₉ x T ₂ , L ₁ x T ₂	L ₁₁ x T ₃ , L ₁₃ x T ₁ , L ₁₅ x T ₃ , L ₁₄ x T ₂ , L ₁₀ x T ₁	L ₁₁ x T ₃ , L ₁₃ x T ₁
		E ₂	L ₁ x T ₁ , L ₁₁ x T ₃ , L ₁₁ x T ₃ , L ₉ x T ₂ , L ₁ x T ₂	L ₈ x T ₁ , L ₆ x T ₃ , L ₁₄ x T ₁ , L ₄ x T ₁ , L ₁₅ x T ₃	Nil
		E ₃	L ₉ x T ₂ , L ₁₃ x T ₃ , L ₁₅ x T ₃ , L ₁₃ x T ₁ , L ₁₃ x T ₃	L ₁₅ x T ₃ , L ₁₁ x T ₃ , L ₂ x T ₃ , L ₁₃ x T ₃ , L ₈ x T ₁	L ₁₃ x T ₃ , L ₁₅ x T ₃ , L ₁₃ x T ₃
5.	Number of fruits per plant	E ₁	L ₄ x T ₃ , L ₁₁ x T ₃ , L ₁ x T ₃ , L ₉ x T ₃ , L ₁₃ x T ₃	L ₂ x T ₁ , L ₈ x T ₁ , L ₇ x T ₃ , L ₁₄ x T ₃ , L ₅ x T ₂	Nil
		E ₂	L ₁₁ x T ₃ , L ₁₁ x T ₂ , L ₄ x T ₃ , L ₁ x T ₃	L ₂ x T ₁ , L ₁₂ x T ₂ , L ₅ x T ₂ , L ₁ x T ₃ , L ₆ x T ₁	L ₁ x T ₃
		E ₃	L ₉ x T ₃ , L ₁₃ x T ₃ , L ₁ x T ₃ , L ₁₁ x T ₂ , L ₁₄ x T ₃	L ₂ x T ₁ , L ₃ x T ₁ , L ₇ x T ₃ , L ₁₄ x T ₃ , L ₁₁ x T ₂	L ₁₁ x T ₂ , L ₁₄ x T ₃
6.	Fruit Length	E ₁	L ₃ x T ₃ , L ₁₃ x T ₂ , L ₁₃ x T ₃ , L ₁₀ x T ₁ , L ₉ x T ₃	L ₁₃ x T ₂ , L ₉ x T ₃ , L ₁₄ x T ₁ , L ₁₁ x T ₃ , L ₃ x T ₃	L ₃ x T ₃ , L ₁₃ x T ₂
		E ₂	L ₃ x T ₃ , L ₉ x T ₃ , L ₁₃ x T ₂ , L ₃ x T ₂ , L ₁₄ x T ₁	L ₉ x T ₃ , L ₁₁ x T ₃ , L ₁₃ x T ₂ , L ₁₄ x T ₁ , L ₇ x T ₁	L ₉ x T ₃ , L ₁₃ x T ₂ , L ₁₄ x T ₁
		E ₃	L ₃ x T ₃ , L ₁₃ x T ₂ , L ₁₁ x T ₃ , L ₉ x T ₃ , L ₃ x T ₂	L ₉ x T ₃ , L ₁₁ x T ₃ , L ₁₄ x T ₁ , L ₇ x T ₁ , L ₁₃ x T ₂	L ₁₁ x T ₃ , L ₉ x T ₃
7.	Fruit girth	E ₁	L ₆ x T ₂ , L ₃ x T ₂ , L ₁₄ x T ₂ , L ₃ x T ₁ , L ₁₄ x T ₁	L ₆ x T ₂ , L ₇ x T ₂ , L ₈ x T ₁ , L ₁ x T ₂ , L ₅ x T ₂	L ₆ x T ₂
		E ₂	L ₃ x T ₂ , L ₁₅ x T ₂ , L ₁₄ x T ₂ , L ₁₄ x T ₁ , L ₄ x T ₂	L ₁ x T ₂ , L ₁₄ x T ₁ , L ₁₃ x T ₃ , L ₈ x T ₁ , L ₂ x T ₃	L ₁₄ x T ₁
		E ₃	L ₃ x T ₂ , L ₁₄ x T ₂ , L ₁₄ x T ₁ , L ₁₅ x T ₂ , L ₅ x T ₂	L ₅ x T ₂ , L ₁₂ x T ₁ , L ₁ x T ₂ , L ₇ x T ₂ , L ₁₄ x T ₁	L ₁₄ x T ₁ , L ₅ x T ₂
8.	Days to harvesting	E ₁	L ₁₁ x T ₃ , L ₉ x T ₁ , L ₁₃ x T ₁ , L ₉ x T ₂ , L ₈ x T ₁	L ₁₁ x T ₃ , L ₁₄ x T ₂ , L ₁₃ x T ₁ , L ₁₀ x T ₁ , L ₁₅ x T ₃	L ₁₁ x T ₃ , L ₁₃ x T ₁
		E ₂	L ₁₁ x T ₃ , L ₁₃ x T ₁ , L ₁ x T ₂ , L ₁₁ x T ₂ , L ₁₃ x T ₂	L ₈ x T ₁ , L ₄ x T ₁ , L ₆ x T ₃ , L ₁₅ x T ₃ , L ₁₄ x T ₁	Nil
		E ₃	L ₁₅ x T ₃ , L ₁₃ x T ₃ , L ₁₁ x T ₃ , L ₉ x T ₂ , L ₁ x T ₁	L ₁₅ x T ₃ , L ₈ x T ₁ , L ₁₁ x T ₃ , L ₁₀ x T ₂ , L ₂ x T ₃	L ₁₅ x T ₃ , L ₁₁ x T ₃
9.	Fruit weigh	E ₁	L ₁₁ x T ₁ , L ₁ x T ₃ , L ₉ x T ₂ , L ₁ x T ₂ , L ₉ x T ₁	L ₂ x T ₃ , L ₁₁ x T ₁ , L ₇ x T ₂ , L ₁₃ x T ₁ , L ₁ x T ₃	L ₁₁ x T ₁ , L ₁ x T ₃
		E ₂	L ₁ x T ₃ , L ₁₁ x T ₂ , L ₁₁ x T ₃ , L ₉ x T ₃ , L ₄ x T ₃	L ₁₅ x T ₁ , L ₁ x T ₃ , L ₁₃ x T ₁ , L ₆ x T ₂ , L ₇ x T ₂	L ₁ x T ₃
		E ₃	L ₉ x T ₂ , L ₂ x T ₃ , L ₁ x T ₃ , L ₄ x T ₃ , L ₁ x T ₂	L ₂ x T ₃ , L ₁₁ x T ₁ , L ₁₉ x T ₂ , L ₃ x T ₃ , L ₁₃ x T ₁	L ₉ x T ₂ , L ₂ x T ₃
10.	Yield per plant	E ₁	L ₁ x T ₃ , L ₉ x T ₃ , L ₁₃ x T ₂ , L ₃ x T ₃ , L ₁₁ x T ₂	L ₃ x T ₃ , L ₂ x T ₁ , L ₁ x T ₃ , L ₁₂ x T ₂ , L ₂ x T ₃	L ₁ x T ₃ , L ₃ x T ₃
		E ₂	L ₉ x T ₁ , L ₄ x T ₂ , L ₁ x T ₂ , L ₁₃ x T ₁ , L ₄ x T ₁	L ₉ x T ₁ , L ₁ x T ₂ , L ₁₃ x T ₁ , L ₂ x T ₃ , L ₃ x T ₃	L ₉ x T ₁ , L ₁ x T ₂ , L ₁₃ x T ₁
		E ₃	L ₇ x T ₃ , L ₁₂ x T ₃ , L ₄ x T ₃ , L ₁ x T ₃ , L ₁₄ x T ₂	L ₃ x T ₁ , L ₇ x T ₃ , L ₂ x T ₁ , L ₄ x T ₃ , L ₁₃ x T ₂	L ₇ x T ₃ , L ₄ x T ₃

Symbol used in the tables

Symbol	Name of line	Symbol	Name of Tester
L ₁	Heritage Green	T ₁	Parbhani Kranti
L ₂	GO-2	T ₂	Arka Abhay
L ₃	BO-2	T ₃	Arka Anamika
L ₄	Punjab Padmini		
L ₅	Harbhajan		
L ₆	Nirmal-303		
L ₇	CO-3		
L ₈	Pusa Sawni		
L ₉	Swati-10		
L ₁₀	Swati- 25		
L ₁₁	Ankur-40		
L ₁₂	VRO-5		
L ₁₃	VRO-6		
L ₁₄	Ratnaraj		
L ₁₅	Varsha Uphar		

were significant for all the characters in all the environments except due to lines for first effective fruiting node. The mean squares due to lines x tester were significant for all the traits in all the environments except for plant height in E₁, E₂ and E₃ and for fruit girth in E₂. The σ^2 GCA effects due to lines and testers revealed that both the lines and testers contributed for various characters in different environments. Estimates of σ^2 SCA effects were greater than σ^2 GCA effects due to lines and testers for all characters in all the environments, which suggested the role of non-additive gene action in the inheritance of most of the characters. The preponderance of non-additive gene action was also reported by (Singh and Singh, 1978), (Arora, 1993), (Sood and Sharma, 2001) and Prakash *et al.* (2002). Recurrent selection could be used for the improvement of these characters. Out of ten traits under study, the negative gca and sca effects were estimated for three characters viz. days to 50 percent flowering, days to harvesting and height of first effective fruiting node were considered desirable, since these traits are negatively correlated with fruit yield per plant. However, positive estimates of gca and sca effects for the remaining traits were considered desirable. The estimates of 15 female lines and 3 male testers for 10 characters (Table 2) indicated that parents

An overall appraisal of GCA effects of the parents (lines and testers) used in the present study indicated that, in general, none of the parent was a good general combiner for all the traits studied. Estimates of GCA effects showed that line L₉ was a good general combiner over the environment as it showed significant GCA effects in favourable direction for varying sets of 6, 7 and 4 characters in E₁, E₂ and E₃, respectively followed by line L₁, L₄, L₁₁ and L₁₃. Among testers, T₁ was good combiner for a set of three characters in E₁, two in E₂ and four in E₃ including

yield per plant except in E₂ environment.

A perusal of SCA effects among hybrids revealed that highest magnitude of positive SCA effects for yield per plant was revealed by L₃ x T₃ in E₁; L₉ x T₁ and L₁ x T₂ in E₂ and L₃ x T₁ in E₃ environment. None of the cross combination exhibited consistently high SCA effects for all the traits studied. The crosses showing high SCA effects did not always involve parents with high GCA effects suggesting that the interallelic interactions are important for the concerning characters. These findings are in agreement with the findings of Pratap *et al.* (1981 a), Poshia and Shukla (1986), (Patel, 1988), (Poshiya, 1992), (Shinde *et al.*, 1995) and (Pawar *et al.*, 1999).

Besides yield, crosses L₃ x T₃ and L₉ x T₁ also revealed desirable SCA effects for number of branches per plant in E₁ and height of first effective fruiting node in E₁ and E₃. Likewise, cross L₁ x T₂ showed high positive SCA effects for number of fruits per plant in all the environments; and cross L₃ x T₁ for fruit weight in E₁ and E₂ environment.

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Micropropagation and *in-vitro* conservation of elite genotype of cactus pear (*Opuntia ficus indica* Mill.)

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Abstract

Micropropagation techniques of cactus pear for a thorn-less, vegetable type elite genotype, which was collected under germplasm collection programmes, was developed successfully through single bud explant. Physiologically mature buds on cladodes were collected and single bud segment was used for culture initiation. Maximum multiple shoots (8 shoots/explant) formation was achieved on MS medium supplemented with 30 g sucrose, 8 g agar and 2 mg 6-Benzylaminopurine (BA) + 0.1 mg alpha-Naphthalene acetic acid (NAA) per liter. Multiple shoot clump were further subjected to culture for shoot elongation medium devoid of plant growth regulators. The elongated shoots were rooted cent per cent under *in vitro* conditions. The rooted plantlets were successfully acclimatized under three step hardening procedure. Under *in vitro* conservation study, *in vitro* rooted plantlets were used for further conservation by reducing growth of the plantlets and by minimizing sub-culturing process. The rooted plantlets were transferred on agar solidified MS medium supplemented with activated charcoal 3 g/l and different combinations of BA (0, 0.5, 1.0 mg/l) and NAA (0, 0.5, 1.0 mg/l). The higher concentration of BA (1.0 mg/l) was found to increase shoot proliferation and was detrimental to root growth of the *in vitro* plantlets whereas higher concentration of NAA (1.0 mg/l) was found to influence both shoot and root growth positively. This technique of *in vitro* conservation was found effective in maintaining cultures continue for more than six years. Further, proliferated growth of *in vitro* maintained plantlets were harvested and transferred to new containers for increasing number of stock materials.

Key words: Micropropagation, *in-vitro* conservation, Cactus pear

Introduction

Cactus pear (*Opuntia ficus indica* Mill.) belongs to family Cactaceae has specialized photosynthesis mechanism known as Crassulacean Acid Metabolism (CAM) and high water use efficiency (WUE) even in areas with annual rainfall as low as 120-150 mm. It is well adapted plant of Indian dessert and largely utilized as bio-fencing to protect vegetable farm or garden from wild animals and other herbivores by the farmers. Moreover, this crop is gradually gaining popularity as fruit and vegetable crops in hot arid regions of the world. Both spine and spineless cultivars are available in Mexico, but most of the Indian genotypes are thorny. The developed Nations such as Mexico, Chile, and America have recognized this crop as fruit, vegetable, animal feed and industrial raw material (Nobel, 1995). This crop is also commercially used in cosmetics and dye industry. Visualizing the horticultural, industrial and commercial potential of cactus pear, Food and Agriculture Organization has successfully implemented a cactus pear

network with the aim to promote cactus pear as an important fruit crop world wide (Pimienta *et al.*, 1993). In this regard efforts were made in India in introduction, collection and conservation of both indigenous and exotic genotypes (Nath *et al.*, 1999). As a results of intensive efforts made at CIAH, Bikaner, about 35 genotypes having elite characteristics have been conserved in field repository. Some of the promising elite genotypes identified, now require large number of propagules for further research and extension purposes. Conventionally, cactus pear is propagated either through seed or cladodes; however, both methods are either not desirable or inefficient (Malda *et al.*, 1999). Vegetative method of multiplication through cladodes also suffers seriously with fungal infection of foot rot disease which leads to poor survival of plantlets (Mauseth, 1979). Therefore, there is an urgent need for propagation of cactus pear through tissue culture technique to build up large population of plant materials of superior and desired genotypes. Although, micropropagation has been successfully attempted for several members of Cactaceae family (Johnson and Emino 1979; Mauseth, 1979; Escobar *et al.* 1986; Clayton *et al.* 1990 and Khalafalla *et al.* 2007). However, no attempts were made for large scale multiplication

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of thornless cactus pear. Further, conservation of elite type genotypes is a prerequisite for improvement of crops. Some cactus genotypes which are spineless and of vegetable types are severely damaged by various insects pests, herbivores and diseases. Therefore, safe conservation technology viz., *in vitro* conservation is also highly desirable for sustainable and efficient resource management of germplasm. Keeping in view the above reasons, the present investigation was planned with the objective of faster multiplication and *in-vitro* conservation of superior genotypes of vegetable type cactus pear for its utilization in arid agro ecosystem of India.

Materials and methods

(A) Micro propagation study

Preparation of mother plant and collection of explants

Selected genotype of Cactus pear (*Opuntia ficus indica* Mill.) was maintained in green house irrigated and fertilized as per general recommendation with proper plant protection measures. Actively growing buds from cladodes were collected and single bud segment with adjacent tissues were used for culture initiation.

Explant preparation and sterilization

The segments of cladodes containing single bud (areole) of 2 x 2 cm size were collected from mother plants and subjected to washing in running tap water and rinsed in mild concentration of detergents followed by gentle rinsing in tap water. Thereafter, explants were treated with 0.1% HgCl_2 for 5-7 minutes. The surface sterilized explant were thoroughly washed several times with sterile water under laminar flow hood and sized to 1 x 1 cm before inoculation.

Culture media and conditions for cladodes induction

Surface sterilized explants were inoculated vertically on 30 ml Murashige and Skoog (1962) MS medium supplemented with 30 g sucrose, 8.0 g agar and various concentration of BA (0, 1, 2 and 4 mg/l) alone or in combination of NAA 0.1 mg/l. The pH of the medium was adjusted to 5.7 and sterilized by autoclaving at 121°C and 15 lbs for 15 minutes. The cultures were incubated at $26 \pm 2^\circ\text{C}$ temperature and 16 hrs photoperiod provided by cool fluorescent tube with 2000 lux light intensity.

Culture media for rooting

After third subculture period the multiple shoot clumps were subjected for elongation to hormone free medium for three weeks. Thereafter, microshoots were transferred to rooting medium containing MS medium supplemented with 30 g of sucrose, 8.0 g agar, 3 g activated charcoal and varied levels of NAA and IAA at concentration of (0, 0.5, 1.0 or 2.0 mg/l). The cultures were incubated at $26 \pm 2^\circ\text{C}$ temperature and 16 hrs photoperiod provided by cool fluorescent tube with 2000 lux light intensity.

Hardening of *in-vitro* produced plantlets

Under arid agro-ecosystem where both low and high temperatures are extreme, atmospheric humidity is very low and wind speed is very high, thus hardening and acclimatization of plantlets are very challenging component of micropropagation. The hardening of *in vitro* produced plantlets were taken under three step hardening procedure i.e. i) Transfer of plantlets to acclimatization hood inside the culture room. ii) Transfer of plantlets in environment control green house. iii) For further growth and development in glass house equipped with intermittent fogging device, different combination of potting mixture such as vermiculite, coco peat or vermi compost, were tried for survival and acclimatization of plantlets to pots.

(B) In vitro conservation study

Rooted plantlets developed under micropropagation study were used for further investigation of *in vitro* conservation, maintenance, growth inhibition and subsequent survival components. Micropropagated cladodes were transferred to MS medium with 8 g agar 3 g activated charcoal supplemented with 0.0, 0.5, 1.0, mg BA and 0, 0.5, 1.0 mg NAA/l for maintenance of the culture and growth inhibition. The observations on different parameters such as shoot and root growth, percentage of vitrified cultures and promotive/inhibitory effect on root growth were recorded after six month of culture incubated for *in vitro* conservation. The cultures were incubated at $26 \pm 2^\circ\text{C}$ temperature and 16 hrs photoperiod provided by cool fluorescent tube with 1000 lux light intensity.

Results and discussion

Effect of BA and NAA on the multiple shoot induction :

Observation recorded with respect to morphogenetic responses of cladode explant to medium supplemented by BA alone or in combination with NAA are summarized in Table 1. Explant inoculated on medium devoid of BA or NAA alone did not show any response. However, different level of BA and NAA produced a remarkable effect on the percentage of explant with shoots. The mean maximum percentage of explants with shoot (80%) were obtained with higher concentration of BA 4 mg/l either alone or in combination of NAA 0.1 mg/l. All concentrations of BA with higher concentration of NAA 1 mg/l found a decreasing influence on the shoot induction.

A perusal the data revealed that the different levels of BA and NAA resulted in significant variation on number of days taken for axillary bud breaking in inoculated cladode explant under *in vitro* condition. Higher concentration combined treatment of BA and NAA resulted in earliest bud break. Initially after first subculture performed after incubation of culture for four weeks period. There was no difference in induction of number of shoots among

Table 1. Effect of BA and NAA on the multiple shoot induction in cactus pear

Treatments			Days taken for bud break	Percentage of explant with shoot	No. of shoot/explant after I subculture	No. of shoot/explant after III subculture	Shoot length (cm)
	BA	NAA					
T ₁	0.0	0.0	00	00	0.0	0.0	0.0
T ₂	1.0	0.0	35.1	70	1.0	6.2	3.4
T ₃	2.0	0.0	32.4	75	1.0	7.1	3.1
T ₄	4.0	0.0	30.2	80	1.0	7.6	2.0
T ₅	0.0	0.1	00	00	0.0	0.0	0.0
T ₆	1.0	0.1	30.4	70	1.0	7.1	3.1
T ₇	2.0	0.1	28.1	80	1.0	8.6	2.9
T ₈	4.0	0.1	26.5	80	1.0	8.1	2.5
T ₉	0.0	1.0	00	00	0.0	0.0	0.0
T ₁₀	1.0	1.0	24.5	20	1.0	3.2	1.9
T ₁₁	2.0	1.0	22.1	30	1.0	3.8	1.7
T ₁₂	4.0	1.0	19.4	38	1.0	4.2	1.1
SEm.			0.29			0.223	0.14
C.D.			0.81			0.623	0.382

Table 2. Effect of NAA and IAA on the rooting of microshoot of cactus pear

Treatment	Concentration (mg/L)	MS Media Rooting	No. of roots (%)
T ₁	Control 0.0	33.33	1.7
T ₂	NAA 0.5	80.00	2.2
T ₃	NAA 1.0	100.00	4.4
T ₄	NAA 2.0	66.67	4.6
T ₅	IAA 0.5	60.00	2.2
T ₆	IAA 1.0	73.33	2.4
T ₇	IAA 2.0	100.00	3.1
SEm.			0.22
C.D.			0.613

all treatment of both BA and NAA. However, there was remarkable difference in number of shoot per explant after third subculture. The treatment without BA or with only NAA failed to induced shoot in the explants. Whereas, in all the treatments of BA alone or in combination with NAA produced shoots which were varied to different treatments. The differentiation of multiple shoots was maximum (8.6 shoots/explant) after third subculture with T₇ treatment (2 mg/l BA + 0.1 mg/l NAA). The influence of T₇ treatment was at par with T₈ treatment. Except the treatment T₁₀, T₁₁ and T₁₂, all treatments were found to produce rootable size of microshoot. This may be due to the effect of higher concentration of NAA in these treatment. All the concentrations of BA alone or in combination of NAA produced promotive influence axillary bud breaking which gave rise to differentiation of multiple shoots in the cladode explants. These results are in agreement with the findings of Escobar *et al.* (1986), Mohamed-Yaseen *et al.* (1995), Giusti *et al.* (2002) and Garcia-Saucedo *et al.* (2005). The less percent-

age of explants with shoots was observed with treatment higher level of NAA 1 mg/l in combination of BA. These results are in close conformity of the results obtained by Khalafalla *et al.* (2007) who observed no improvement in shoot multiplication when NAA 0.5 mg/l used in combination of BA on kinetin in cactus. Thus, the synergetic influence of NAA at higher concentration did not improve the number of shoots per explant. This may be due to higher concentration of NAA with respect to BA must have suppressed stimulative effect of BA.

Effect of NAA and IAA on rooting in microshoots

The data given in Table 2 show effect of NAA and IAA on rooting percentage and number of roots in microshoot when transferred to MS medium containing different concentration of NAA and IAA. The mean maximum rooting percentage (100%) was obtained in T₃ and T₇ treatments. However, significantly higher number of roots (4.4) was recorded with NAA 1 mg/l.

In the present experiment, it was observed that rooting percentage in microshoots was better with NAA 1 mg/l. Auxin mediated induction of roots in cacti is well documented. *In vitro* studies by Johnson and Emino (1979), Mohmed-Yasseen *et al.* (1995) and Garcia-Saucedo *et al.* (2005) also support the present findings. These results indicate that the higher concentration of auxins have better stimulative influence on root initiation whereas lower concentration exhibited delayed rooting which may be due to in-sufficient quantity for root induction. Similar observations were reported by Mata-Rosas *et al.* (2001) with Mexican cactus *Turbinicarpus laui* and Jaurez and Passera (2002) with *Opuntia ellisiana* Griff. Zeng *et al.* (2003) obtained root formation with MS medium + 0.1 mg IBA l⁻¹ after 10 days of culture with *Opuntia dillenii* cv. *Milpa Alta*.

Acclimatization of the plantlets

In vitro propagated plantlets were successfully acclimatized by transferring them into small pots of plastic, containing potting mixture of different medium. The rate of survival of the plantlets (Table 4) in the pots was 100% with vermiculite + cocopeat mixture of 3:1 (v/v) which was followed by another mixture i.e. vermiculite + vermicompost (60%). The lowest survival of plantlets (50%) observed with two step procedure of hardening. The higher percentage of survival of plantlets with vermiculite along with either cocopeat may be due to the complementary good properties of vermiculite and cocopeat in terms of porosity, high cation exchange capacity (CEC), highly decomposed, stable and good water holding capacity. Preece and Sutter (1991) also emphasized that certain environmental conditions are necessary for acclimatization of plantlets using controlled environmental facilities.

Similar achievements (100% survival of plantlets) were reported by Johnson and Emino (1979), Vyaskot and Jara (1984), Mohamed-Yasseen *et al.* (1995), Giusti *et al.*

(2002) and Garcia-Saucedo *et al.* (2005).

In vitro conservation of plantlets

Under *in vitro* conservation study, *in vitro* rooted plantlets were used for further conservation by reducing growth of the plantlets and by minimizing subculturing process. The effect of BA, NAA and their combinations on the *in vitro* growth, injury and survivability of the cultures was evaluated. The rooted plantlets were transferred on agar solidified MS medium supplemented with activated charcoal 3g /litre and different combinations of BA (0, 0.5, 1.0 mg/l) and NAA (0, 0.5, 1.0 mg/l). The higher concentration of BA (1.0 mg/l) was found to increase shoot proliferation and was detrimental to root growth of the *in vitro* plantlets whereas higher concentration of NAA (1.0 mg/l) was found to influence both shoot and root growth positively (Table 3). This technique of *in vitro* conservation was found effective in maintaining cultures continue for more than six years. Further, proliferated growth of *in vitro* maintained plantlets was harvested and transferred to new containers for increasing numbers of stock materials. Addition of auxin in the medium had optimal positive influence on shoot and root growth. This is due to physiological action of NAA in cell division and stimulation of apical dominance in the micro-shoots and adventitious root formation including cell elongation. The study conducted on auxin induced morphogenetic responses in long-term *in vitro* subcultured *Mammillaria san-angelensis* Sanchez-Mejorada (Cactaceae) by Rubluo *et al.* (2002) and concluded that auxin may be involved in *in vitro* morphogenetic response in Cactaceae. Further, these results may be supported by the findings of Sajid *et al.* (2006) who studied the effect of diverse hormonal regimes on *In vitro* growth of grape germplasm.

Table 3. Effect of BA, NAA and their combination on the *in vitro* growth, injury and survivability of the cultures

Treatments	BA	NAA	Increase in Shoot length cm.	Increase in Root length cm.	Percentage of vitrified culture	Root growth promotive/inhibitory effect
T ₁	0.0	0.0	1.0	1.0	33.33	Promotive
T ₂	0.0	0.5	2.6	1.6	13.33	Promotive
T ₃	0.0	1.0	3.1	2.6	13.33	Promotive
T ₄	0.5	0.0	1.1	0.0	40.00	Inhibitory
T ₅	0.5	0.5	0.5	0.0	53.33	Inhibitory
T ₆	0.5	1.0	0.4	0.0	53.33	Inhibitory
T ₇	1.0	0.0	0.6	0.0	60.00	Inhibitory
T ₈	1.0	0.5	0.5	0.0	73.33	Inhibitory
T ₉	1.0	1.0	0.6	0.0	80	Inhibitory
SEm.			0.102	0.11		
C.D.			0.29	0.32		

Table 4. Influence of hardening procedure on survival percentage of plantlets

Hardening step	Survival % of plantlets	
	Vermiculite : Cocopeat (3:1)	Vermiculite : Vermicompost (3:1)
Two step procedure: Transfer to Environment control greenhouse for 8-10 days and subsequent transfer in shade house equipped with intermittent fogging.	80	50
Three step procedure: Primary hardening 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 equipped with intermittent fogging.	100	60

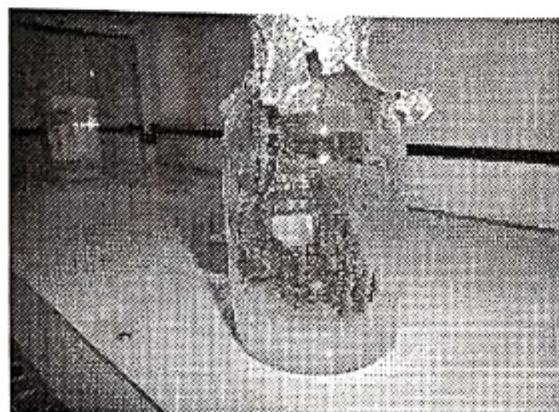


Fig. 1. Micropropagated cladode of Cactus pear

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Evaluation of rhizobial isolates from pigeonpea nodules for characterization of fast growing antagonist of *Fusarium udum*

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Abstract

Eight isolates of rhizobia were isolated from pigeonpea nodule and identified and characterized by physiological and biochemical tests. Isolate KCC5 showed maximum potential antagonistic characters. Phylogenetic analysis of 16S rDNA sequences of KCC5 with other reference strains. Form this analysis, KCC5 aligned to same cluster (100% bootstrap value) with reference strains of *Sinorhizobium fredii*. All the strains of *Pseudomonas* spp. were positive to IAA production and phosphate solubilisation. Only KCC2 and KCC5 were produced siderophore. KCC5 produced maximum quantity of siderophore. In dual culture technique, maximum inhibition of *F. udum* recorded in the case of KCC5. Cell free culture filtrate of KCC5 also restricted the hyphal growth of *F. udum*. Morphological abnormalities in the hyphae of *Fusarium udum* obtained from the zone of interaction during dual culture were observed by light microscopy. Fragmentation and degradation of mycelia and loss of structural integrity of conidia of *F. udum* were clearly observed under scanning electron microscopic analysis. In iron supplemented (30 μ M Fe³⁺) medium inhibition of the pathogenic fungi was reduced. It was found that under iron-deficient condition KCC5 inhibited maximum vegetative growth of *F. udum* as compared to under iron-sufficient conditions.

Key words: *Fusarium*, *Rhizobium*, *Pseudomonas*, phosphate solubilisation, IAA production.

Introduction

Pigeonpea (*Cajanus cajan*) is one of the important pulse crops and a very popular food in developing tropical countries. India is a principal pigeonpea-growing country contributing nearly 90% of total world's production and it is widely grown in almost all the states. *F. udum* is the most important soil-borne disease of pigeon pea causes fusarial wilt. Biological control of *F. udum* has attracted the attention throughout the world. In general, now-a-days, the idea of controlling soil borne plant pathogens including *F. udum* with chemical pesticides or fungicides has been shifted to the option that biological control can have an important role in agriculture. The beneficial effect of *Rhizobium* in legumes in terms of biological nitrogen fixation has been a main focus in the recent years. Obviously, rhizobia are known to increase nodulation and nodule weight in legumes along with increase in host plant growth and development besides, Rhizobia are reported to inhibit several soil-borne phytopathogenic fungi including *Macrophomina phaseolina* (Perdomo *et al.*, 1995;

Arora *et al.*, 2001), *Fusarium oxysporum* (Antoun *et al.*, 1987; Nautiyal, 1997), *Rhizoctonia bataticola* and *Pythium* sp. (Nautiyal, 1997). The antagonistic activities of rhizobia are mainly due to the production of secondary metabolites such as siderophores (Rioux *et al.*, 1986; Arora *et al.*, 2001; Deshwal *et al.*, 2003), antibiotics (Breil *et al.*, 1996), HCN (Beauchamp *et al.*, 1991; Antoun *et al.*, 1998), and phytoalexin production (Chakraborty and Chakraborty, 1988; Deshwal *et al.*, 2003).

The present study investigates the diversity of fast growing rhizobia of pigeonpea nodules based on variations in partial sequences of the 16S rRNA gene, analysis of morphological, physiological, biochemical data, assessment of secondary metabolite production and antagonism against *Fusarium udum*.

Materials and methods

Rhizobium isolates

Pigeonpea nodulating bacteria were isolated from nodules of pigeonpea plants grown in farmers field of Betul district (M.P.), India in July and August. This area comes under a temperature range of 18°C (in winters) to 41°C (in

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summers). For the isolation of the root nodulating bacteria, method of Vincent (1970) was followed. *C. cajan* seedlings were uprooted carefully and root nodules were collected and washed with the sterile distilled water followed by surface treatment with 95 % ethanol (2 ml) and again rinsed with sterile distilled water. Washed nodules were surface sterilized quickly (2-3 min) with 0.1% mercuric chloride (HgCl_2) and again cleaned for at least 6 times with sterile distilled water so as to remove the traces of HgCl_2 . The nodules were crushed in a half filled culture tube with saline water (0.85% NaCl) with the help of sterile glass rod. A milky bacterial suspension obtained was serially diluted and streaked on pre-incubated yeast extract mannitol (YEM) agar (Vincent, 1970) plates. Plates were incubated at $28 \pm 1^\circ\text{C}$ for 4-5 days and observed for specific features of rhizobia. In total eight isolates were obtained from *Cajanus cajan* which were maintained separately on YEM agar slants at 4°C , for further. These were named as KCC (*Cajanus cajan* rhizobia).

Phenotypic characterization of isolated bacteria

Morphological and physiological characteristics were determined for eight test isolates and the type strains of *Sinorhizobium meliloti*. Gram's staining of the isolates was done to provide information on presumptive tests of isolates. Colony morphology of nodule isolates (colony color, colonial form, elevation and colonial margins) were determined on YEM agar plates containing congo red dye using standard bacteriological techniques (Vincent, 1970). For the biochemical tests of log phase cultures (10^8 cells ml^{-1}) of root nodule isolates were used. They were separately inoculated in YEM broth as well as on YEMA and incubated at 28°C . All the tests were carried out in triplicates and at least three times. Motility was examined following hanging drop method. Generation time of each isolates was measured. The isolates (single colony of each) were inoculated in 50 ml of YEM broth in conical flasks and incubated at rotary shaker (150 rpm at $28 \pm 1^\circ\text{C}$) for 48 h. The growth (turbidity produced) was measured by O.D. method by O.D. at 610 nm after every 6 h in UV-VIS spectrophotometer (Shimadzu, Model UV-1601). The generation time was calculated by following formula: $\text{Generation Time} = (T_2 - T_1) / 3.3 (\log \text{OD}_2 - \log \text{OD}_1)$, where, $(T_2 - T_1)$ is time interval taken at any two points in the log phase of growth and $(\log \text{OD}_2 - \log \text{OD}_1)$ is difference between the log values of OD at time T_2 h to $\log$ value of OD at time T_1 h. Growth on/in glucose peptone agar (GPA) (Kleczkowska *et al.*, 1968), Hofer's alkaline broth (HAB) (Hofer, 1935), 2% NaCl (Sadowsky *et al.*, 1983), calcium glycerophosphate (Hofer, 1941), catalase activity (Graham and Parker, 1964), Oxidase Activity (Kovaks, 1956) gelatin hydrolysis (Sadowsky *et al.*, 1983), hydrolysis of urea (Lindstrom and Lehtomaki, 1988), citrate utilization (Kosar, 1923), in presence of KNO_3 (El-Idrissi *et al.*, 1996), acid production (Arora *et al.*, 2000), hydrogen sulfide production (Zobell and Feltham, 1934), MR-VP test, utilization of carbon sources (El-Idrissi *et al.*,

1996), poly hydroxy butyrate (PHB) accumulation (Navarini *et al.*, 1992) were checked for each isolated bacteria.

Isolation of fungal pathogen

The cropping areas of Badgaon, Betul district in Madhya Pradesh, India were surveyed and wilt prone area were marked. Samples of diseased root parts and seeds were randomly collected and fungal pathogen *Fusarium udum* was isolated from infected roots of pigeon pea (*Cajanus cajan*) following the water agar technique (Musket and Malone, 1941). This technique was used for the isolation of external and internal mycoflora of the diseased seeds and infected roots showing reddish brown lesions on the hypocotyls. The external surface mycoflora was isolated by directly placing the seeds and infected plant material on the water agar medium containing 2% agar and penta-chloro-dinitrobenzene (PCNB). For the isolation of internal mycoflora, seeds and diseased roots were surface sterilized by soaking them in 0.5% sodium hypochlorite solution for 3-5 min. Samples were then rinsed in several changes of sterile water to remove the traces of sodium hypochlorite, and placed on the surface of pre-incubated water agar plates. After incubation at 28°C for one week, plates were observed for the appearance of various fungi.

Identification of fungal pathogens

Different fungal isolates from infected seeds and plant materials were grown on the potato dextrose agar (PDA) medium. The fungal cultures were maintained on potato dextrose agar (PDA) medium and characterized following illustration and description of standard mycological literature and 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 recorded and compared with those as described by Booth (1971). Isolated fungal cultures of *F. udum*, with their known cultures from IIPR, Kanpur, was maintained on PDA medium at 4°C .

IAA production

To observe IAA production exponentially grown cultures (10^8 cells ml^{-1}) of the strains were incubated separately on broth medium for 24 h. Supernatant of the strains were collected by centrifugation at 10,000 rpm for 15 min at 4°C and 2 ml supernatant of each was transferred separately to a fresh tube to which 100 μl of 10 mM O-phosphoric acid and 4 ml of Salkowski reagent (1 ml of 0.5 mM FeCl_3 in 35% HClO_4) were added. The mixture was incubated at room temperature for 25 min, and the absorbance of pink colour developed was read at 530 nm using UV-VIS spectrophotometer (Shimadzu, Model UV-1601). The concentration of IAA in cultures was determined by using a calibration curve of pure IAA as a standard following the linear regression analysis.

Phosphate Solubilization

Phosphate solubilization ability of isolated strains was detected by spotting them separately on Pikovskya's agar plates. Plates were then incubated at $28 \pm 1^\circ\text{C}$ for 3 d, and observed for the clearing zone around the colonies (due to the solubilization of inorganic phosphate by bacteria). The quantitative estimation of phosphate solubilization was carried out after modified method of Jackson, (1958). Exponentially grown (10^8 cells ml^{-1}) cultures were incubated in Pikovskya's broth supplemented. After 48 h, supernatants were collected by centrifugation of cultures passing through $0.45 \mu\text{m}$ millipore filter membrane. Observations were made by comparing with the standard curve in the same conditions.

Siderophore production

Siderophore production was determined on Chrome-azurol S (CAS) medium following the method of Schwyn and Neilands (1987). For the preparation of CAS assay solution, 6 ml of 10 mM HDTMA solution was diluted upto 100 ml with double glass distilled water and a mixture of 1.5 ml iron (III) solution (1 mM $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ in 100 mM HCl) and 7.5 ml of 2 mM aqueous CAS solution was slowly added under stirring. Anhydrous piperazine (4.307 g) was dissolved in water and 6.25 ml of 12 M HCl was carefully added to it. This buffer solution (pH 5.6) was rinsed into the volumetric flask which was then filled with water to afford 100 ml of CAS assay solution. The CAS shuttle solution was obtained by adding 5-sulfosalicylic acid to the above solution at a concentration of 4 mM. The bacterial strains (24 h old cultures) spotted separately on CAS medium. Plates were incubated at $28 \pm 1^\circ\text{C}$ for 48 h. Formation of orange to yellow halo around the colonies showed the production of siderophore. Presence of hydroxamate type of siderophores was determined following the method of Gibson and Magrath (1969) 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 un-inoculated 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 pro-

duction, 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.

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 $50 \mu\text{g ml}^{-1}$ 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 $100 \mu\text{l}$, 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'-CCGAATTCGTCG-ACAACAGAGTTTGATCCTGGCTCAG3' 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 $50 \mu\text{l}$ reaction mixture included 5-10 ng of bacterial DNA as template, $1 \mu\text{l}$ of each primer, 1U of *Taq* DNA polymerase (Bangalore Genei, India) and $100 \mu\text{M}$ 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 f1 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/phree-reduced.html). Phylogenetic tree obtained with bootstrap values in cluster algorithm, phylip format and topological algorithm

Antifungal activities of *Sinorhizobium fredii* KCC5

Antagonistic properties of *Sinorhizobium fredii* KCC5 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 (10^8 ml⁻¹) of KCC5 was spotted 2 cm juxtaposed from the fungal disc and incubated at $28 \pm 1^\circ\text{C}$ 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 28°C for 7 days. After incubation broth culture of the strains were collected by centrifugation at 10,000 rpm for 15 min at 4°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 28°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 *S. fredii* KCC5 was made under iron-deficient and iron-sufficient (30 and 60 μM Fe³⁺) 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 vigor-

ously 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/inch² (121°C) for 20 min. Antagonism was carried out by following the methodology mentioned as before. Bacterial broth supplemented with 30 and 60 μM Fe³⁺ 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 CO₂ 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

All the isolates of pigeonpea nodules were characterized by morphological, physiological and biochemical means (Table 1). All the isolates (KCC1 to KCC8) were Gram-negative, non-sporing, and non-capsulated

Table 1 Morphological, physiological and biochemical characters of root nodulating bacteria from *Cajanus cajan* var. Manak isolates

Characteristics	KCC 1	KCC 2	KCC 3	KCC 4	KCC 5	KCC 6	KCC 7	KCC 8	MTCC 99	MTCC 2378	MSSP	MTCC 100
Gram reaction	-	-	-	-	-	-	-	-	-	-	-	-
Growth at 28°C	+	+	+	+	+	+	+	+	+	+	+	+
Growth at 41°C	-	-	-	-	-	-	-	-	-	-	-	-
Motility	+	+	+	+	+	+	+	+	+	+	+	+
Capsule	+	+	-	-	+	+	+	-	+	+	+	+
Endospore	-	-	-	-	-	-	-	-	-	-	-	-
PHB accumulation	+	+	+	+	+	+	+	+	+	+	-	+
Generation time (h)	2.6	2.5	2.8	2.5	2.6	2.4	2.5	2.5	2.7	2.9	2.6	2.4
Catalase	+	+	+	+	+	+	+	+	+	+	-	+
Oxidase	+	+	+	+	+	+	+	+	+	+	+	+
H ₂ S	-	-	-	+	-	-	-	+	-	-	-	-
Gelatin hydrolysis	-	-	-	-	-	-	-	-	+	+	-	+
Starch hydrolysis	-	-	-	-	+	-	-	-	-	-	-	+
Citrate utilization	+	+	+	+	+	+	+	+	+	+	+	+
Growth on GPA	+	+	+	+	+	+	+	+	-	-	+	-
Growth in HAB	-	-	-	-	-	-	-	-	+	+	-	+
2% NaCl tolerance	+	+	+	+	+	+	+	+	+	+	+	+
8% KNO ₃ tolerance	-	-	-	-	-	-	-	-	+	+	-	+
Ca-glyc	-	-	-	-	-	-	-	-	+	+	-	+
Utilization of:									+	+	-	+
Arabinose	+	+	+	-	-	+	+	+	+	+	+	+
β alanine	-	+	+	+	+	+	+	-	-	+	+	-
Sucrose	+	+	+	+	+	+	+	+	+	+	+	+
Glucose	+	+	+	+	+	+	+	+	+	+	+	+
Mannitol	+	+	+	+	+	+	+	+	+	+	+	+
meso-Inositol	-	+	+	+	+	+	+	+	+	+	+	+
Lactose	+	+	-	+	+	+	-	-	+	+	+	+

Abbreviations: +, positive; -, negative; GPA- glucose peptone agar; HAB- Hoffer's alkaline broth; PHB- poly hydroxyl butyrate; Ca- glyc, precipitation in Calcium glycerophosphate; *Sinorhizobium meliloti* MTCC-100; *Rhizobium leguminosarum* MTCC-99; *Mesorhizobium loti* MTCC-2378; *Burkholderia* sp. MSSP (Pandey and Maheshwari, 2005)

which were motile with single sub-polar flagellum. The isolates formed red, semi-translucent, rounded, smooth, mucoid colonies on CrYEMA with 2 to 4 mm diam after 48 h of incubation.

All the isolates were fast growers with average mean generation time of 2.9 h showing catalase and oxidase activities and produced yellow colour to bromothymol blue indicated acid production. Almost all the isolates were positive for urease and gelatinase production, utilized citrate and precipitated calcium glycerophosphate. Except KCC5, all the isolates were negative for starch hydrolysis. Isolate KCC5 was negative for urease, while KCC4 was unable to utilize citrate and arabinose. The isolates failed to grow on GPA but were able to grow in HAB and could tolerate 8% KNO₃ as well as 2 % NaCl (Table 1). All the isolates utilized dextrose, glucose and glycerol when the carbon sources replaced mannitol in YEMA. Isolates KCC1 and KCC2 were unable to utilize cellobiose and KCC2 and KCC3 were negative for lactose utilization. The characters of isolates were compared with standard rhizobial strains viz., *Sinorhizobium fredii* MTCC-100, *Rhizobium leguminosarum* MTCC-99 and *Mesorhizobium loti* MTCC-2378 and most of them possessed characteristics similar to *S. fredii* MTCC-100 (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, In-

dia) and observed that total length of amplicons was approximately 1500 bp (1.5 kb) which represented the amplification of nearly full length 16S rRNA gene from all the *Cajanus cajan* isolates. The size of the PCR product corresponded with the size of the 16S rRNA genes among all eight isolates.

Neighbour-joining dendograms were generated (based on cluster algorithm and topological algorithm) using 16S rDNA sequences of *cajanus cajan* root nodulating isolates and representative rhizobial sequences from EMBL/Gen Bank/DDBJ/PDB (Fig. 4). The 16S rDNA sequence of the following strains (type strains unless otherwise indicated) were obtained from GenBank: *Sinorhizobium fredii* S8 (EF506208), *Sinorhizobium fredii* S25 (EF506206), *Sinorhizobium fredii* S42 (EF506204). A neighbour-joining dendogram was generated using the sequence from *Rhizobium* sp. KCC5 and representative *Sinorhizobium fredii* sequences from GenBank (Fig. 5). From this analysis, KCC5 aligned to same cluster (100% bootstrap value) with other reference strains (Fig. 5). This analysis has revealed that *Cajanus cajan* nodulating isolate KCC5 can be named now as *Sinorhizobium fredii* KCC5.

Except KCC4 all strains of *Rhizobium* spp. (KCC 1 to KCC8) were positive for IAA (indole acetic acid) production as evidenced by the development of pink colour of the cell free supernatants (Table 2). *Rhizobium* sp. KCC5 produced maximum IAA and its production was induced in

Table 2 Plant growth-promoting and antifungal properties of *Rhizobium* strains

Strains	IAA ^A	Phosphate solubilization ^B	HCN ^C	Siderophore ^D	Antagonism against <i>F. udum</i>
KCC 1	+	++	-	-	-
KCC 2	-	+	-	-	+
KCC 3	+	+	-	+	+
KCC 4	+	+	-	+	+
KCC 5	++	+	-	+++	++
KCC 6	+	+	-	+	+
KCC 7	+	+	-	+	+
KCC 8	-	+	-	-	-
Standard strains					
MTCC 100	+	+	-	-	-
MTCC 99	-	+	-	-	-
MTCC 2378	-	+	-	-	-

Abbreviations: A -, IAA negative, +, IAA positive; B -, Phosphate solubilization negative; +, phosphate solubilization positive, C -, HCN negative, +, HCN positive; D -, 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; *Sinorhizobium meliloti* MTCC-100; *Rhizobium leguminosarum* MTCC-99; *Mesorhizobium loti* MTCC-2378

presence of tryptophan. In KCC5, the IAA production was started after 18 h of inoculation. The IAA production increased corresponding to incubation period and reached maximum after 168 h of incubation (Fig. 2). During phosphate solubilization all strains of *Rhizobium* spp. formed clear halos around their spot inoculation by solubilizing tricalcium phosphate on the Pikovskya's agar. All strains solubilized inorganic phosphate (Table 2A). Lowest pH recorded was 3.8 subjected after 7 d incubation. No acidic phosphatase activity was recorded. Phosphate solubilization was started after 24 h of incubation in all the strains and maximum in *Rhizobium* sp. KCC5 at 7th d of incubation (Fig. 2B). None of the strains of *Rhizobium* spp. was able to produce cyanogens (Table 2). All the strains were screened for siderophore production on CAS agar, *Rhizobium* sp. KCC2 and *Rhizobium* sp. KCC5 showed

siderophore activity as revealed by orange halo around their colonies (Table 2). Secretion of siderophore by both the strains started after 12 h of incubation. On further incubation up to 5 days, siderophore production got increased but later on production got declined. To know the type of siderophore, 48 h old supernatant of the strain (*Rhizobium* sp. KCC5) scanned at 400 nm showed major peak. This peak corresponded with hydroxamate type of siderophore. None of the strain was able to develop wine colour in the reaction mixture following the methods of Arnow (1937) which ruled out the possibility of catechol-phenolic type siderophore.

All the *Rhizobium* strains were screened for antagonistic activity against *Fusarium udum*. Only *Rhizobium* sp. KCC2 and *Rhizobium* sp. KCC5 inhibited the growth of both pathogenic fungi on PDA plates at 28°C

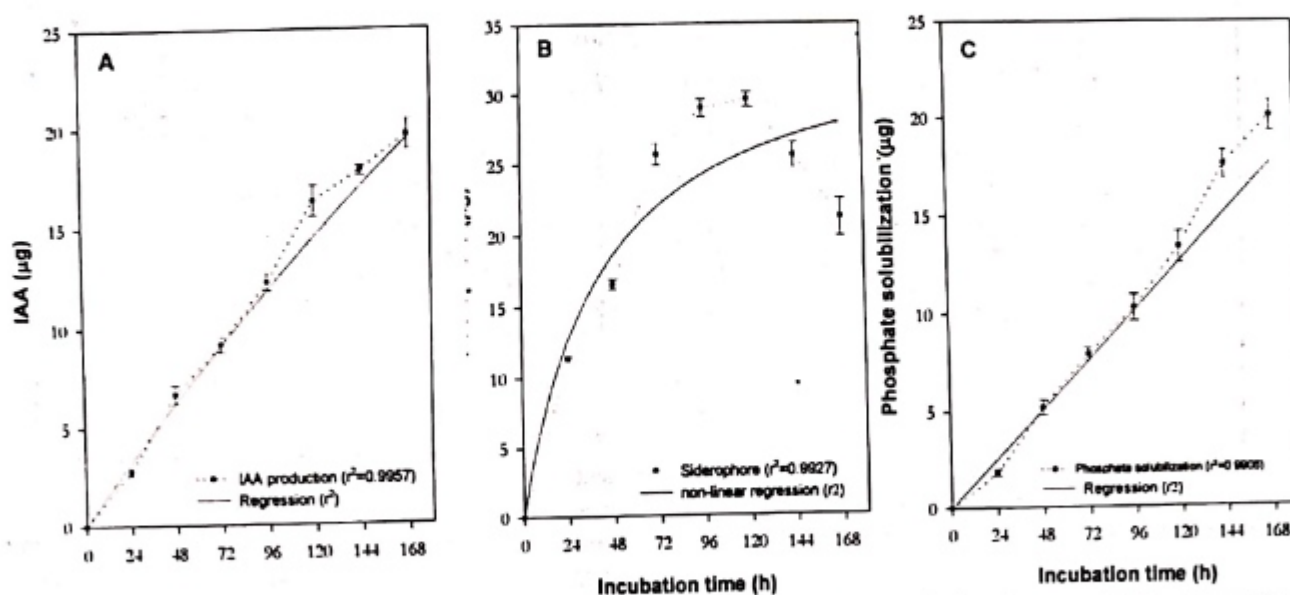


Fig 1. Time course of IAA production (A), siderophore production (B) and phosphate solubilization (C) by *Sinorhizobium fredii* KCC5 (C) (Values are means of standard deviation of three replications)

(Table 2). Increase in fungal inhibition corresponded to incubation period. Strain KCC5 was able to inhibit 56 % of *F. udum* after 6 days of incubation. (Fig. 3). None of the standard strains *Sinorhizobium meliloti* MTCC-100, *Rhizobium leguminosarum* MTCC-99, and *Mesorhizobium loti* MTCC-2378 inhibited *F. udum* (Table 2). Cell free culture filtrates of *Sinorhizobium fredii* KCC5 also restricted development of *F. udum*. It was observed that fungal inhibition by *Sinorhizobium fredii* KCC5 was more pronounced in dual culture conditions in comparison to that of cell free culture filtrates (Fig. 3).

To find out the siderophore-mediated inhibition of *F. udum*, *Sinorhizobium fredii* KCC5 was examined to inhibit it on modified YEM medium supplemented with 30 and 60 μM Fe^{+3} . In this experiment, the mycelial growth of the pathogen significantly got decreased by the antagonist with respect to control in iron-deficient conditions. Inhibition of the pathogen was reduced in iron supple-

mented (30 μM Fe^{+3}) medium. However, inhibition was less pronounced on the 60 μM Fe^{+3} supplemented medium in comparison to normal conditions. *Sinorhizobium fredii* KCC5 showed 56% inhibition of *F. udum* in 30 μM Fe^{+3} supplemented medium (Fig. 4) after 6 days of incubation. Under iron-deficient conditions remarkable inhibition of *F. udum* was observed and under such prevailing conditions, *Sinorhizobium fredii* KCC5 inhibited 48% vegetative growth *F. udum*, respectively (Fig. 4).

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* were observed under light microscopy (Fig 5). Fragmentation and degradation of mycelia and loss of structural integrity of

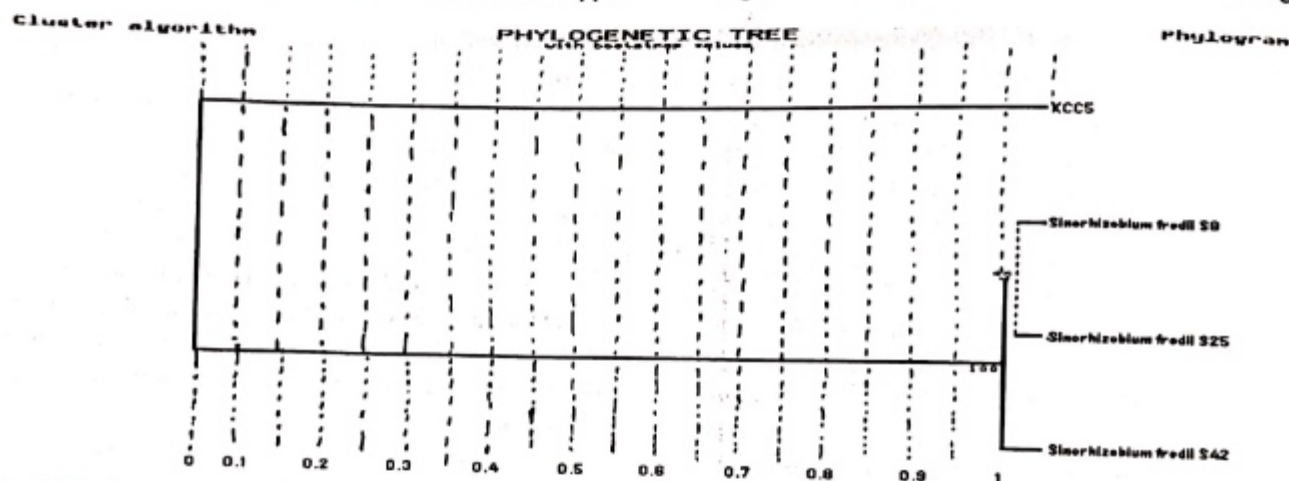


Fig 2. Phylogenetic relationship between bacterial strains of *Rhizobium* and representing species (A) and *Rhizobium* sp. KCC5 and representing species (B) based on partial length 16S rDNA sequences constructed using cluster algorithm. Bootstrap values are indicated (from 1000 replication). Accession numbers are mentioned in text.

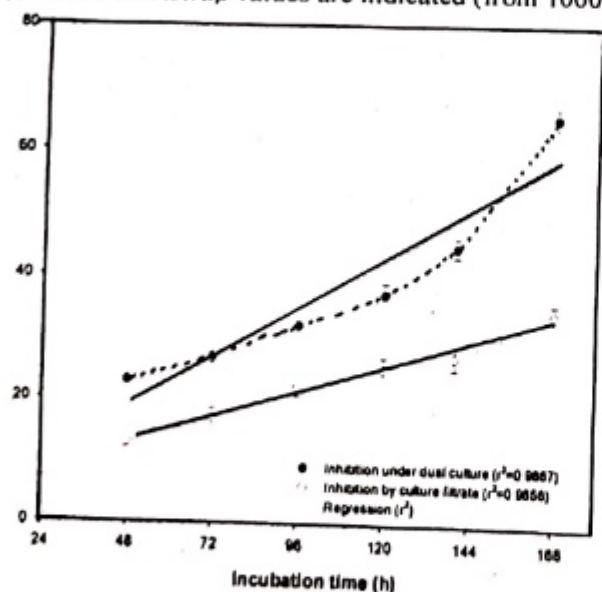


Fig 3. Inhibition of *Fusarium udum* by *Sinorhizobium fredii* KCC5 under dual culture and by Cell free culture filtrate (Values are means of standard deviation of three replications)

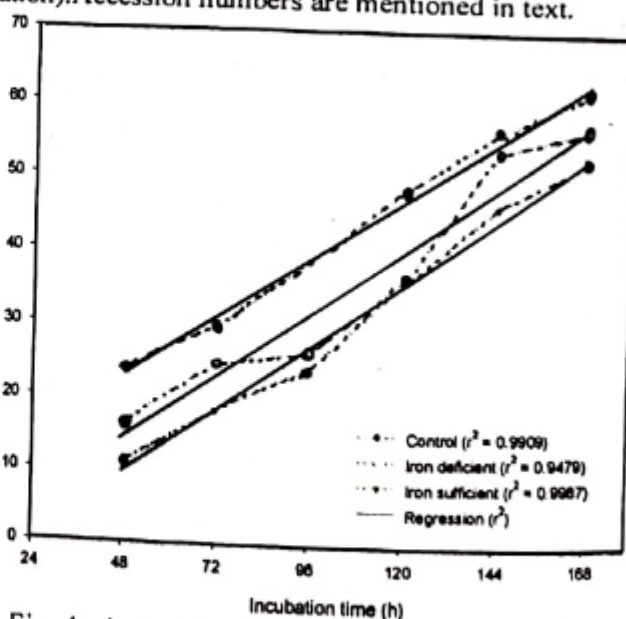


Fig 4. Antagonism between *Fusarium udum* and *Sinorhizobium fredii* KCC5 under iron sufficient and iron deficient conditions (Values are means of standard deviation of three replications)

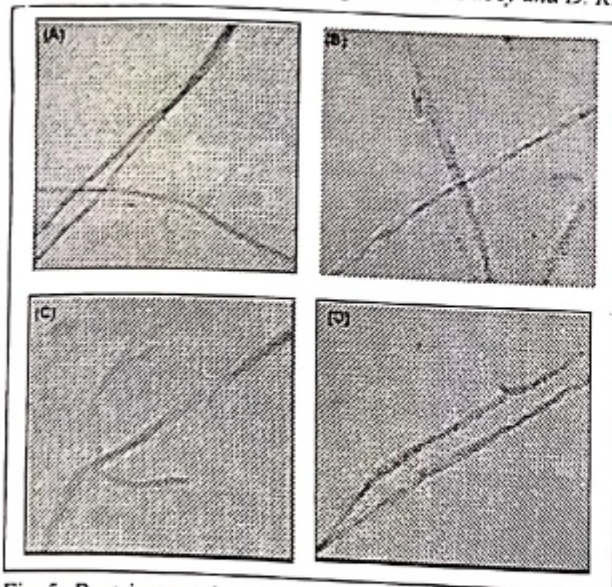


Fig 5: Post interaction events in *Fusarium udum* due to *Sinorhizobium fredii* KCC5. control (A) hyphal degradation (B) halo cell formation, hyphal perforation and digestion of fungal cell wall (C) cytoplasm coagulation (D) (see arrows)

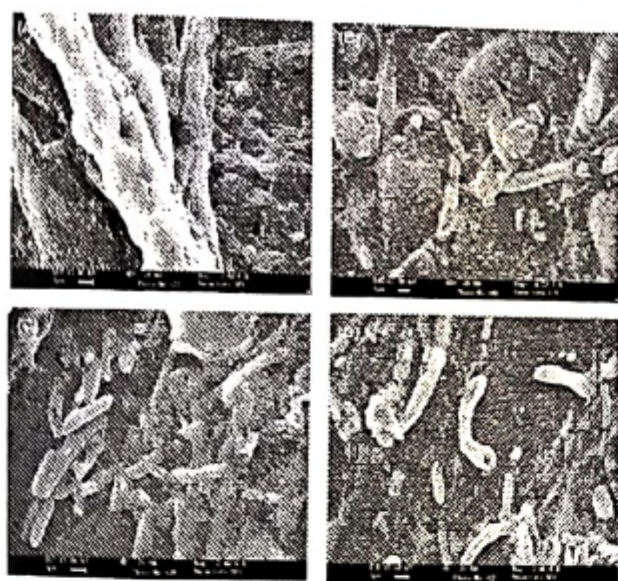


Fig 6: Scanning electron microscopic photographs showing mycelial and conidial destruction by antagonistic interaction effects of *Sinorhizobium fredii* KCC5; hyphal lysis (A and B), lysis of conidia (C and D) (see arrows)

conidia of *F. udum* were clearly observed under Scanning electron microscopic analysis (Fig 6). These features of mycelia may be due to production of siderophore by strains *Sinorhizobium fredii* KCC5.

Discussions

Rhizobia are α -proteobacteria able to establish symbiotic interactions with leguminous plants leading to the formation of root nodules where they fix atmospheric

nitrogen. All the isolates from *Cajanus cajan* were fast growing with average generation time 2.6 h. All isolates of from *Cajanus cajan* were Gram-negative, aerobic, non-spore forming and cocci in shape (Holt *et al.*, 1994). Colony morphology of *Rhizobium* has been demonstrated to play an important role in process of symbiotic nitrogen fixation (Vincent, 1972). Most of the strains were positive for catalase and oxidase activity and utilized citrate. The strains precipitated calcium glycerophosphate. All strains failed to grow on GPA but able to grow in HAB and tolerated 8% KNO₃ and 2% NaCl. The characters reported in the present study of all bacterial strains are similar to those as described by Holt *et al.*, (1994).

The strains were able to utilize wide range of carbohydrates including hexoses, pentoses, disaccharides and trioses. This is also in accordance with the earlier findings that sinorhizobia with other fast growing rhizobia are capable of growing on variety of carbon substrates (Jordan, 1984; Elkan, 1992). Further, the morphological and physiochemical characters of the strains were found to be similar to that of genus *Sinorhizobium* genera Holt *et al.*, (1994).

Out of eight strains of *Rhizobium* spp. (KCC1 to KCC8) isolate KCC5 was found most potential antagonist against *F. udum*. Therefore, it was further characterized by 16S rDNA sequence homology. In Phylogenetic tree based on 16S rDNA homology *Rhizobium* sp. KCC5 is placed in the *Sinorhizobium fredii* clade. The use of rDNA gene analysis has become promising. Polymerase chain reaction (PCR) amplification of rDNA genes from environmental DNA samples, combined with fingerprinting techniques such as denaturing gradient gel electrophoresis (DGGE), terminal restriction fragment analysis (T-RFLP), amplified rDNA restriction analysis (ARDRA), cloning and sequencing, provide detailed information about the species composition of whole communities (Nicol *et al.*, 2003; Torsvik and Ovreas, 2002). 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. Recently, Pandey *et al.* (2005) have also characterized *Burkholderia* spp. MSSP from root nodules of *Mimosa pudica* on the basis of 16S rDNA analysis.

Siderophore production by root nodulating rhizobial strains has been well documented (Smith *et al.*, 1985 and Carson *et al.*, 2000). Various strains of rhizobia have been reported to produce a wide range of siderophores such as rhizobactin, citrate, catechol, anthranilate under iron deficient conditions (Smith *et al.*, 1985; Guerinet *et al.*, 1990 and Deshwal *et al.*, 2003). In the present study, *Sinorhizobium fredii* KCC5 produced hydroxamate type of siderophore. Earlier, Arora *et al.* (2001) and Deshwal *et al.* (2003) reported hydroxamate and catechol type siderophores from *S. meliloti* RMP1 and

Bradyrhizobium (Arachis) sp. respectively. Hydroxamate type of siderophore production appears to be confined to *R. leguminosarum* as reported earlier by Patel *et al.* (1988) and in *Bradyrhizobium* by Guerinot (1991). Gibson and Magrath, (1969) stated the presence of hydroxamate type of siderophore in majority of rhizobia. Probably this was due to study of limited host range of leguminous plants (Young, 2003). In rhizobia, the ability to synthesize siderophores is restricted to a limited range of strains, rather than wide distribution (Carson *et al.*, 2000). These results were also complementary with the study of Arora *et al.* (2001) who found only 20% of rhizobial isolates produced siderophores.

Inhibitory effect of *S. fredii* KCC5 was observed against *F. udum* under iron sufficient conditions. It was found that inhibitory activity of strains *S. fredii* KCC5 was strongly 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 $20\mu M$ (Lynch, 1990), optimal at $5-10\mu M Fe^{+3}$ and inhibited beyond this concentration (Neilands, 1981). In the present study, KCC5 examined for inhibition of *F. udum* under iron ($30\mu M Fe^{+3}$) supplemented conditions showed reduction in inhibition against both the fungal pathogens. On increasing the concentration of iron to $60\mu M Fe^{+3}$, more inhibition was recorded in comparison to normal iron condition. Similar findings were obtained by Derylo and Skorupska (1993) who reported that $60\mu M Fe^{+3}$ 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 *S. fredii* KCC5. Similarly, reduced inhibitory activity of rhizobacteria due to siderophores was observed under iron rich conditions as supported by Lynch (1990).

Microscopic abnormalities in fungal pathogens under the influence of *S. fredii* KCC5 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 *S. fredii* KCC5. 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.

In vitro, fungal growth inhibition assay by *S. fredii* KCC5 revealed significant reduction in radial growth of *F. udum*. Inhibition zones between the test organisms viz., *S. fredii* KCC5 against *F. udum* mainly due to production of 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). Antagonistic strains of *Rhizobium* sp. (Perdomo *et al.*, 1995), *B. japonicum*

(Deshwal *et al.*, 2003), *S. meliloti* (Arora *et al.*, 2001) inhibited *M. phaseolina* and its disease incidence in different crop plants. Inhibition of *Fusarium oxysporum* causing wilt disease in chickpea have also been reported by the involvement of *Rhizobium* sp. NBR19513 (Nautiyal, 1997) and *S. meliloti* (Antoun *et al.*, 1998). *S. fredii* KCC5 showed inhibition of *F. udum* in cell free culture filtrates. Different bacteria imparted varying degrees of inhibition of growth and conidia germination of phytopathogenic fungi due to production of antifungal compounds in culture filtrates (Skidmore and Dickinson, 1976; Upadhyay and Rai, 1987). 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. Isolate of pigeonpea nodule, *S. fredii* KCC5 have been proved a great potential in controlling phytopathogenic fungus *F. udum*. Further work is in progress on interaction studies for preparation of combination of two or more than two biocontrol agents for better control of *F. udum*.

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Influence of soil moisture conservation practices on soil properties under arid conditions of Rajasthan

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Abstract

A field experiment was conducted during 2004-09 to study the effect of soil water conservation practices viz., FYM mulch (@ 30 t/ha), cluster bean (*Cyamopsis tetragonoloba*) straw mulch (@ 8 t/ha), bui (*Aerva pseudotomentosa*) grass straw mulch (@ 8 t/ha), sub-surface application of FYM (@ 12 t/ha) on soil properties. The soil samples were collected after the harvest of brinjal crop in January each year. The pooled data of five years were analysed for various soil physicochemical properties. Among various soil moisture conservation practices application of FYM mulch was found superior than others. It improved soil hydraulic conductivity from 29.35 in control to 27.12 cm h⁻¹, soil moisture content at field capacity from 3.30 to 3.88% w/w, wilting point from 1.20 to 1.54% w/w, organic carbon from 0.38 to 2.41 g kg⁻¹, available N from 78 to 120 kg ha⁻¹, P from 8.16 to 13.74 kg ha⁻¹, K from 430 to 621 kg ha⁻¹, available Fe from 4.1 to 7.1 mg kg⁻¹, Mn from 4.5 to 6.5 mg kg⁻¹, Cu from 0.13 to 0.34 mg kg⁻¹ and Zn from 0.08 to 0.30 mg kg⁻¹.

Key words: Soil properties, organic mulch, farmyard manure, sandy soil and arid region

Introduction

Soil and water conservation are the two most important aspect for management of light textured sandy soils. These soils are characterized by high leaching rate of irrigation water and nutrient from the root zone of the crop. To improve the water and nutrient level in the soil the addition of farmyard manure (FYM) in the crop fields is a common practice because FYM directly adds much of organic carbon (Duiker and Lal, 1999) and other nutrients to the soil. The method of FYM application or other residues incorporation as organic mulch can play a great role in improving the micro-climate of soil and enhancing the supply of nutrients. These organic mulches also directly affect the microclimate around the plant and in the subsurface region of soil by modifying the radiation budget of the surface, add nutrient to the soil, improve moisture holding capacity and hydraulic conductivity of soil, reduce crusting by avoiding the direct impact of rain drops on the soil particle and decreasing the soil water loss (Gupta and Gupta, 1985; Gupta, 1989; and Singh *et al.*, 2006). The decrease in soil water evaporation results in more uniform soil moisture content and increasing the efficiency of irrigation water, which is of paramount importance in dry areas. Therefore, the present investigation was conducted to find out the influence of organic mulches on change in soil properties in arid condition.

Material and methods

Field trials were conducted from July 2004 to June 2009 on very deep, coarse loamy sand Typic Torripsamments (sand 86%, silt 5%, clay 8%) at the research Farm of the Central Institute for Arid Horticulture, Bikaner, India, located between 27° 11' to 29° 3' N latitude and 71° 54' to 74° 12' E longitude with a mean annual rainfall of 250 mm with brinjal (eggplant) (*Solanum melongena* L.) as the test crop. The region is characterized by high wind velocity coupled with high evaporation rates, high temperature and solar radiation, low and irregular distribution of rainfall and generally experience water deficit during plant growth period. The soil was calcareous, alkaline in reaction single grain structure having bulk density 1.58 Mg m⁻³; soil moisture retention capacity ranged from 3.30% w/w at field capacity and 1.20% at wilting point for 0.0 to 15.00 cm depths; saturated hydraulic conductivity 29.35 cm h⁻¹; pH (1:2.5 soil water suspension) 8.2; EC 0.07 dS m⁻¹; soil organic carbon (SOC) 0.38g kg⁻¹, total N 0.0021%; available N 78 kg ha⁻¹; available (Olsen) P₂O₅ 8.16 kg ha⁻¹; and available K₂O 430 kg ha⁻¹; available Fe 4.1; Mn 4.5; Cu 0.13; and Zn 0.08 mg kg⁻¹.

The experiment was laid out in a randomized block design with three replications of four soil water conservation treatments, viz., FYM mulch, cluster bean (*Cyamopsis tetragonoloba*) straw mulch, bui (*Aerva pseudotomentosa*) grass straw mulch, sub-surface application of FYM at 20 cm depth and control. All the treatments were imposed after 15 days of transplanting in the month of July each year. The plot sizes were 5.0 m x 1.5 m. The FYM and straw

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mulches were applied @ 30 and 8 t ha⁻¹, respectively. The sub-surface application of FYM was done @ 12 t ha⁻¹. The N (Urea), P₂O₅ (di-ammonium phosphate) and K₂O (muriate of potash) were applied @ 80, 60 and 40 kg ha⁻¹, respectively.

After the harvest of brinjal crop in the month of January each year, the soil samples were collected from each treatment at 0-15 cm depth and analysed for pH, EC, CaCO₃, organic carbon, available N, P, and K following their standard methods as described by Jackson (1973). Available micronutrients i.e., Fe, Mn, Cu and Zn in soil samples were extracted with 0.005 M DTPA (Lindsay and Norvell, 1978) and analysed by atomic absorption spectrophotometer. Soil bulk density was determined using core method (Blake and Hartge, 1986) and moisture retention with a pressure plate method (Richards, 1954). The saturated hydraulic conductivity was determined by constant head method (Klute, 1965).

Results and discussion

Soil properties

Physical properties

There was no change in texture, structure, bulk density and particle density of soil due to application of soil water conservation practices. However, a significant change in porosity, water holding capacity and hydraulic conductivity of soil was recorded (Table 1). FYM mulch and FYM subsurface incorporation treatments were found significantly effective in increasing the soil moisture retention capacity both at field capacity and wilting point. Pertaining to the status of hydraulic conductivity of soil, all the treatments recorded significant improvement in hydraulic conductivity as compared to control. The results are in consonance with the findings of Blevin *et al.* (1983). They reported that straw mulching had no effect on bulk density of soil. Mulching studies conducted in the inter-row spaces of cowpea (*Vigna unguiculata*, Cv. FS-68) with native weeds @ 6 t ha⁻¹ increased the infiltration rate by 10-20% and moisture retention (at -0.01 MPa) by 6.0-20 per cent (Gupta, 1986).

Table 1. Physical properties of soils (0-15 cm) after five years of experimentation (Pooled data of five years from 2004 to 2009).

Treatments	Bulk density Mg m ⁻³	Particle density Mg m ⁻³	Porosity (%)	Soil moisture at F.C. w/w (%)	Soil moisture H.C. w/w (%)	Sat. at W.P. cm h ⁻¹
FYM mulch	1.57	2.77	44.04	3.88	1.54	27.12
Cluster bean straw mulch	1.58	2.77	42.96	3.35	1.15	28.45
Grass straw mulch	1.58	2.77	42.96	3.30	0.91	28.76
FYM incorporation	1.58	2.77	42.96	3.58	1.46	28.10
Control	1.58	2.78	43.16	3.30	1.20	29.35
CD at 5%	NS	NS	0.368	0.067	0.184	0.315

Chemical properties

Soil water conservation practices could not bring significant changes in values of pH and EC as compared to control (Table 2). However, CaCO₃ content was significantly higher in the treated plots as compared to control. A slight decline in the value of pH may be attributed to the production of organic acids, phenolic and carboxylic compounds and secretions of growing biomass.

The SOC of these soils is generally low both at surface and subsurface horizons (Table 2). The low content of organic carbon (0.36 to 2.42 g kg⁻¹) in these soils is due to limited biological activity and rapid decomposition of biomass under prevailing arid climatic conditions (Gupta, 1989). The effects were positive and appreciably higher in the FYM mulch treatment. SOC were recorded significantly higher in all the treated plots as compared to the control. The highest SOC (2.42 g kg⁻¹) was recorded in FYM mulch treatments. The result is in agreement with the findings of Duiker and Lal (1999). They reported a positive linear effect of organic mulch application rate on soil organic carbon concentration.

Table 2 presents the pooled data for available N, P and K content in soil. A comparison of available N and P contents of the treated plots with control plots clearly indi-

cates that application of organic mulches results in significant increase in nutrient status of the soils under the present agroclimatic conditions. FYM mulch registered maximum content of available N (120 kg/ha) and P (13.74 kg/ha) of the soils as compared to control showing N and P values of 78 kg/ha and 8.16 kg/ha, respectively. Increase in available N, P and K due to FYM mulching is due to direct addition of NPK through FYM to the available nutrients pool in the soil. The decomposition of organic matter is accompanied by the release of appreciable quantities of carbon dioxide which, when dissolved in water, forms carbonic acid, which is capable of decomposing certain primary minerals (Tolanur and Badanur, 2003). Further, the beneficial effect of FYM on the available K is also due to the reduction of potassium fixation and release of K due to the interaction of organic matter with clay. Significantly higher available N and P were recorded with straw mulching as compared to control. This may be attributed to the reduction in soil temperature due to straw mulching influencing the biological processes (Hartfield and Prueger, 1996) and enhances soil N mineralization (Tian *et al.*, 1993). It has been shown that in calcareous soils, CO₂ production plays an important role in increasing the phosphate availability.

Table 2. Chemical properties of soils of 0-15 cm depth after five years of experimentation (Pooled data of five years from 2004 to 2009).

Treatments	pH	EC (dS m ⁻¹)	CaCO ₃ (%)	Org. C g kg ⁻¹	Avail. N (kg ha ⁻¹)	Avail. P ₂ O ₅ (kg ha ⁻¹)	Avail. K ₂ O (kg ha ⁻¹)	Fe	Mn	Cu	Zn
										(mg kg ⁻¹)	
FYM mulch	8.30	0.08	2.0	2.42	120	13.74	621	7.1	6.5	0.34	0.30
Cluster bean straw mulch	8.20	0.10	2.2	0.68	88	11.56	393	5.0	5.3	0.25	0.20
Grass straw mulch	8.21	0.12	1.8	0.57	89	10.50	352	4.5	4.6	0.24	0.18
FYM incorporation	8.26	0.07	2.5	0.68	95	11.72	453	5.6	5.5	0.28	0.21
Control	8.20	0.07	1.0	0.38	78	8.16	430	4.1	4.5	0.13	0.08
CD at 5%	NS	NS	0.660	0.05	9.320	1.425	9.591	0.218	0.675	0.026	0.041

The data on DTPA extractable micronutrients (Fe, Mn, Cu and Zn) are given in table 2. The initial status of available Zn and Cu contents indicated that soils were deficient in these nutrients because of their coarse texture. Treatments were found to be significantly effective in increasing the available micronutrient status of soil. An increase in micronutrients status was observed in all the treated plots as compared to control. However, Mn was at par in grass mulch treatment and control.

It was concluded that based on improvement in physico-chemical properties of soil FYM mulch was proved to be the best treatment. Cluster bean straw mulch may be another option for improved soil properties.

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Effect of organic and inorganic sources of nitrogen on growth, yield and quality of cabbage (*Brassica oleracea* var. *capitata* L.)

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Abstract

A field experiment to study the Effect of organic and inorganic sources of nitrogen on growth, yield and quality of cabbage was conducted at Horticulture Farm, SKN College of Agriculture, Jobner. Results showed that the plant height, plant spread, number of leaves and leaf area increased significantly with the integrated application of nitrogen as compared to control. The highest values for these parameters were recorded with the application of nitrogen 50% through urea + 50% through vermicompost. The yield increased maximally and significantly when nitrogen was supplied as 50% through urea + 50% through vermicompost. It was also recorded that application of 50% nitrogen through urea + 50% nitrogen through vermicompost registered a significant higher values of growth, yield, nitrogen and protein content in leaves of cabbage. However, 50% nitrogen through urea + 50 % nitrogen through poultry manure was found second most important treatment in increasing the traits under study, which was statistically at par with 50% nitrogen through urea + 50% nitrogen through vermicompos.

Key words: *Brassica Oleracea*, vermicompost, yield

Introduction

Cabbage (*Brassica oleracea* var. *capitata* L.) is one of the most important member of genus *Brassica* grown in world. Golden Acre is very popular variety of cabbage being used by the farmers in the vicinity of big cities owing to its earliness, round and compact head. The productivity of cabbage in India is very low (16.5 tonnes) in comparison to European countries (25.17 tonnes) and it is true with Rajasthan in comparison to other states of India. Available literature reveals that the low soil fertility, nutritional imbalances, adverse climatic conditions, etc. are some of the basic reasons for low productivity of cabbage in Rajasthan.

It is a well known that nitrogen increases the growth and yield of most of the crops, particularly leafy vegetables including the cabbage. Application of nitrogen through inorganic fertilizers can enhance the growth and yield to considerable extent, it adversely affect the fertility and productivity of soil in longer duration. Therefore, it is important to supplement the nitrogen by organic sources like urea. The FYM is rich source of organic matter and able to replenish most of the macronutrients being taken up by crop (Abdel-Nasser and Hussein, 2001). Poultry manure and vermicompost are the concentrated source of nutrients. It is well documented that it is an excellent source of organic matter which increases uptake of several other

nutrients (Abusaleha 1992; Jose *et al.*, 1988). Therefore, it is hypothesized that the yield and quality of cabbage can be enhanced to a great extent by application of nitrogen through a combination of urea with different organic sources of nitrogen besides increasing the soil fertility in a sustainable manner. With this background, the present experiment was conducted to evaluate the effect of organic and inorganic sources of nitrogen on growth, yield and quality of cabbage (*Brassica oleracea* var. *capitata* L.).

Material and Methods

The experiment was conducted on Golden Acre variety of cabbage at Horticulture Research Farm, S.K.N. College of Agriculture, Jobner. The climate of this region is semi-arid characterized by aridity of the atmosphere and extremity of temperature both in summer (45.5°C) and winter (-1°C). The average rainfall of this area is 500 mm, most of which occurs during the period of July to mid September. The soil of experimental site was loamy sand (85.2% sand 9.2% silt and 5.4% clay). The soil having pH 8.0, electrical conductivity 1.20 dSm⁻¹ in (1:2.5) soil water suspension, organic carbon 0.16% and available N, P₂O₅ and K₂O of 130, 15.20 and 140 kg ha⁻¹, respectively.

The experiment was laid out in a Randomized Block Design (RBD) with three replications. The treatments

were comprised of total 17 combinations of urea, vermicompost, FYM and poultry manure including control (no fertilizer). The seeds were first raised in nursery and then transplanted in field after 6 weeks. The distance between plant to plant as well as row to row was kept at 45 cm. N:P:K was applied at the rate of 150:80:75 kg ha⁻¹ respectively as per zonal recommendation. For treatments, nitrogen was supplied through FYM, vermicompost, poultry manure and urea. Full dose of phosphorus, potash, FYM, vermicompost and poultry manure were applied at the time of transplanting, while urea was applied in two split doses i.e. half at the time of transplanting and remaining half dose at 30 days after transplanting. Standard practices were followed to raise the crop.

Five plants were randomly selected in each plot and tagged for observations on various traits like plant height, plant spread, number of leaves per plant, leaf area and average weight, volume and diameter of head and yield at marketable maturity. Among quality attributes nitrogen content, protein content and vitamin 'C' content were analyzed (A.O.A.C., 1960).

Results and discussion

Growth attributes

Plant height, plant spread, number of leaves and leaf area increases significantly in most of the treatments with the application of nitrogen either alone or in combination of organic and inorganic sources. Application of nitrogen 50% through urea + 50% through vermicompost (T_9) recorded the maximum plant height (21.55 cm), plant spread (42.74 cm), number of leaves per plant (22.34) and leaf area (2010.6 cm²). These values were followed by application of nitrogen 50% through urea + 50% through poultry manure (T_{12}). Statistically treatments T_8 and T_{12} were non significant in most of the growth traits (Table 1). Increase in yield and other attributes to the involvement of nitrogen in chlorophyll synthesis, protein synthesis and assimilation of various other organic compounds of physiological significance in the plant system. The organic manures not only provide the plant nutrients, but also improve the physical properties of the soil such as porosity, CEC and WHC, etc. Gopal and Lal (1996) reported that number of leaves and plant height of cabbage cv. Golden Acre increased with increasing levels of nitrogen.

The increase in plant growth with the application of urea in combination with vermicompost might be due to the fact that the urea readily supplied nitrogen in early crop growth stages whereas vermicompost prolonged the supply of nutrients to crop during the entire growing season. Vermicompost might have also improved the physical and biological properties of soil. The physiological parameters pertaining to photosynthesis, stomatal conductance and transpiration also exhibited significant increment with nitrogen supplied through urea in combination with vermicompost. It indicates that the increase in these physi-

ological parameters might be attributed via increased ion absorption from soil, enhanced carbohydrate assimilation and production of new tissues which have ultimately increased vegetative growth. The other sources of nitrogen i.e. FYM & poultry manure in combination of urea found effective in increasing growth of cabbage because of similar properties of soil. Tomar *et al.* (1998) also reported that with the application of vermicompost leaf area of carrot was significantly increased. Similarly, the plant height, root and shoot biomass in tomato were significantly increased when 20% commercial horticulture medium was replaced by vermicompost. These results are in close agreement with Thanunathan *et al.* (1997) in onion and Reddy *et al.* (1998) in garden pea who observed significantly increased values of growth parameters due to application of vermicompost.

Replacement of vermicompost with poultry manure also enhanced the vegetative growth of cabbage. It might be due to the fact that poultry manure contains uric acid having 60 percent nitrogen. The uric acid rapidly changes to ammonia form causing its immediate and efficient utilization for better plant growth and development. Enhanced vegetative growth with the application of urea in combination with poultry manure has also been reported by Jose *et al.* (1988) in brinjal and Wang *et al.* (1996) in cabbage, cucumber and white guard. Jose *et al.* (1988) obtained highest plant height and early flowering in brinjal with the application of nitrogen 50% through urea and 50% through poultry manure. Yadav (2002) also reported the maximum increase in growth and yield attributes in okra with the application of poultry manure added urea. Awodun (2007) also reported that application of poultry manure @6 t/ha increase the growth and yield parameters such as number of leaves, stem girth and length of internodes of fluted pumpkin relative to control.

Yield attributes and yield

Among all the treatment combinations, treatment T_9 (50% N through urea and 50% N through vermicompost) produced the maximum weight, highest diameter and maximum volume of head. These parameters were significantly higher over other treatments except T_{12} (50% N through urea + 50% N through poultry manure). The yield was recorded maximum with application of nitrogen 50% through urea and 50% through vermicompost (T_9) which was higher than other treatments, but statistically at par with the application of nitrogen 50% through urea + 50% through poultry manure (T_{12}). The treatments T_8 and T_{12} registered an increase in yield in the order of 39.52 and 22.33 percent, respectively over control (Table 2).

The significant improvement in yield and yield attributes on account of vermicompost application along with urea might have attributed to the translocation of nutrients from soil, particularly at later stages of crop growth when sink was able to synthesize the enhanced amount of

Table 1. Effect of organic and inorganic sources of nitrogen on plant height, plant spread, number of leaves and leaf area per plant in cabbage.

Treatment		Plant height (cm)	Plant spread (cm)	No of leaves (Per plant)	Leaf area (cm ²)
T ₁	Control	15.20	33.13	17.10	1453.5
T ₂	100% N (urea)	18.65	38.06	20.61	1749.1
T ₃	75% N (urea) + 25% N (FYM)	18.55	36.43	19.65	1719.7
T ₄	50% N (urea) + 50 % N (FYM)	18.35	36.23	19.81	1735.4
T ₅	25% N (urea) + 75% N (FYM)	18.15	34.79	18.53	1649.2
T ₆	100% N (FYM)	17.20	34.42	17.41	1566.9
T ₇	75% N (urea) + 25% N (VC)	18.45	38.36	20.09	1796.9
T ₈	50% N (urea) + 50 % N (VC)	21.55	42.74	22.34	2010.6
T ₉	25% N (urea) + 75% N (VC)	19.20	37.11	19.82	1744.2
T ₁₀	100% N (VC)	18.10	35.56	18.85	1658.8
T ₁₁	75% N (urea) + 25% N (PM)	19.05	35.16	18.38	1619.3
T ₁₂	50% N (urea) + 50 % N (PM)	19.70	40.38	20.85	1876.5
T ₁₃	25% N (urea) + 75% N (PM)	18.15	36.12	19.49	1715.1
T ₁₄	100% N (PM)	17.65	34.27	18.21	1607.9
T ₁₅	50% N (urea) + 25% N (FYM) + 25% N (VC)	19.10	35.85	19.18	1695.9
T ₁₆	50% N (urea) + 25 % N (FYM) + 25% N (PM)	18.70	34.72	19.68	1733.8
T ₁₇	50% N (urea) + 25% N (VC) + 25% N (PM)	19.35	38.60	20.32	1828.6
SEmt		0.67	1.18	0.65	51.22
CD(P=0.05)		1.93	3.39	1.85	147.19

VC= Vermicompost, PM= Poultry manure

Table 2. Effect of organic and inorganic sources of nitrogen on weight, diameter and volume of head and yield in cabbage.

	Treatment	Average head weight (kg)	Average head diameter (cm)	volume (cc)	Yield (q ha ⁻¹)
T ₁	Control	0.616	11.14	0.762	252.16
T ₂	100% N (urea)	0.700	13.03	0.866	276.52
T ₃	75% N (urea) + 25% N (FYM)	0.673	12.79	0.835	273.60
T ₄	50% N (urea) + 50 % N (FYM)	0.662	12.66	0.824	269.84
T ₅	25% N (urea) + 75% N (FYM)	0.649	12.05	0.802	263.50
T ₆	100% N (FYM)	0.631	11.68	0.783	256.45
T ₇	75% N (urea) + 25% N (VC)	0.705	12.92	0.875	292.76
T ₈	50% N (urea) + 50 % N (VC)	0.808	14.76	1.003	351.82
T ₉	25% N (urea) + 75% N (VC)	0.678	12.85	0.847	283.15
T ₁₀	100% N (VC)	0.638	12.17	0.792	265.77
T ₁₁	75% N (urea) + 25% N (PM)	0.693	12.66	0.860	286.91
T ₁₂	50% N (urea) + 50 % N (PM)	0.787	13.46	0.976	308.48
T ₁₃	25% N (urea) + 75% N (PM)	0.659	12.54	0.823	280.09
T ₁₄	100% N (PM)	0.641	11.80	0.791	261.22
T ₁₅	50% N (urea) + 25% N (FYM) + 25% N (VC)	0.661	12.45	0.820	282.88
T ₁₆	50% N (urea) + 25 % N (FYM) + 25% N (PM)	0.642	11.79	0.796	272.35
T ₁₇	50% N (urea) + 25% N (VC) + 25% N (PM)	0.703	12.78	0.871	302.12
	SEmt	0.026	0.527	0.036	15.94
	CD (P=0.05)	0.076	1.514	0.104	45.81

VC= Vermicompost, PM= Poultry manure

carbohydrate assimilated by the enhanced rate of photosynthesis. Further, increased vegetative growth might have provided more sites for translocation of photosynthates, which ultimately resulted in increased yield. Higher rates of photosynthesis, transpiration and stomatal conductance due to application of vermicompost in present investigation also strengthen these findings. These results were in accordance with the findings of Reddy *et al.* (1998) in garden pea. Tomar *et al.* (1998) in brinjal and Oliveira *et al.* (2001) in cabbage. Reddy *et al.* (1998) reported that maximum number of pods per plant, seeds per pod and yield of garden pea cv. FC-1 can be obtained by application of 10 t vermicompost ha⁻¹ along with recommended doses of NPK (27.5:60:50 kg ha⁻¹). Likewise, Oliveira *et al.* (2001) reported that highest head weight (700 g) and yield (38 t ha⁻¹) of cabbage cv Mastukaze was obtained with the application of earthworm compost @ 27 and 29 t ha⁻¹, respectively. Azarmi *et al.* (2008) concluded that addition of vermicompost @ 15t/ha significantly increase the growth and yield of tomato. Premsekhar and Rajashree (2009) also reported that FYM @20 t/ha recorded the highest yield of okra (10.39 t/ha) with the B:C ratio of 3.56.

Quality attributes

Nitrogen content in leaves, protein content in head and vitamin C content were increased with the application of organic sources of nitrogen along with urea as compared to control (Table 3). However, there was no significant effect of organic manures or urea on vitamin C content of the head. Application of nitrogen 50% through urea + 50% through vermicompost exhibited maximum nitrogen content of 3.57% in leaves and protein content of 14.61% in head of cabbage. It might be due to improved nutritional environment in the rhizosphere as well as its utilization in the plant system, leading to enhanced translocation of nutrients, vitamins and proteins in heads. Another reason might be the increased activity of nitrate reductase which helped in synthesis of certain amino acids and proteins as reported by Lopes *et al.* (1996) in cowpea and Rajkhowa *et al.* (2000) in green gram. Yadav (2002) also reported that organic manures in combination with inorganic form of nitrogen increased the protein content in okra. Mukesh Kumar *et al.* showed that total chlorophyll content was increase with the application of poultry manure and vermicompost as compared to urea. Premsekhar and Rajashree (2009) also reported that chlorophyll content in the leaves of okra have been significantly improved with the application of organic sources of nutrients.

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Evaluation of drip irrigation and mulches on the yield and yield attributes of bottle gourd [*Lagenaria siceraria* (Molina) Stendl.]

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Abstract

Water is one of the precious input on which productivity of vegetable crops depends. However the precious input is scarcity available in hot arid areas of Rajasthan. Besides rain water, both surface and ground water being used for vegetable production. The water is applied to the plants either through gravity flow system or pressurized irrigation system. It is estimated that the efficiency of canal irrigation system is about 40% resulting in wastage of water with low application efficiency. Therefore, a field experiment on the use of drip irrigation system with and without mulches was conducted during the year 2006 & 07 in the rainy season at research farm of COA, SKRAU, Bikaner. There were four volume of water and three mulches were used singly and in their possible combination. The maximum length of fruit and number of fruits were observed with the treatment of drip 0.70 V and among the mulches these parameters were higher under organic mulch. The maximum fruit yield was recorded with application of 0.70 V of water and with organic mulch singly and in combined form.

Key words: Drip irrigation, bottle gourd, mulching

Introduction

Rajasthan, though the largest state in the country the area under vegetables in the state is 135682 ha with production of 818900 M tones (Annonymous, 2009). The productivity in the state is 6.0 tones/ha which is very less than the national productivity of 16.1 tones/ha. It is attributed to poor cultural management practices including irrigation management, especially in arid region, where leaching losses of water is very high. Therefore, the root suffers for water deficit and the potential of crop is not expressed in true-to-type. Micro irrigation has got rid-off such problem and it is benefiting option as far as water use economy is concerned.

Water is the most valued agri-input required for successful growth of the plants. Being chief constituent of protoplast, universal solvent and carrier of solutes in plant metabolic system, water is most inevitable input governing the cultivation of any crop. In vegetable crops which are largely herbaceous in nature, water management deserves special significance especially in bottle gourd crop which requires wide spacing. Furthermore, in arid region where water has been scarce input for the production of vegetables for long period, any technique offering water to the plant in efficient manner is a matter of worth consideration. Drip irrigation system, letting in water drop by drop to the plant, has been proved efficacious in harvesting better crop yield at the cost of per unit consumption of

water. In arid regions due to high thermal stress, wind velocity, low and erratic rainfall, moisture losses through evaporation and transpiration is more and affect the much productivity of the vegetables. It has been observed that temperature in the plant root zone during peak summer season (40-45 °C) leading to rapid drying of soil which seriously affect the growth and establishment of the plant. Walkar (1969) reported that even at 1 °C difference in soil temperature could have a significance role on plant growth. The technique of mulching both organic and inorganic is effective in minimizing the water loss from soil surface as a result of solar radiation and wind action. Therefore, the present investigation was carried out to find out the optimum water requirement under drip system in bottle gourd under arid conditions.

Material and methods

The field experiment was conducted during kharif season of 2006 and 2007 at College of Agriculture, Bikaner. The experimental site lies in hyper arid partially irrigated western plain (zone IC) of Rajasthan that comes under M2 agro climatic zone (major Region) and M9E1 (sub region), as per NBSS and LUP. The average annual rainfall is varies from 141 mm to 325 mm. The mean daily maximum goes up to 42°C during summer with relative humidity less than 4 per cent and 23.6°C during winter. The soil having pH 8.4 with percolation rate 9 cm/hour (Singh, 2002).

Further experiment, was undertaken on Hybrid "Ward" which is one of the popular hybrid of bottle gourd. The drip irrigation system consisted of 75 mm and 63 mm main and sub main line, respectively, with lateral (16 mm) spacing 160 cm and dripper (3 lph) spacing 75 cm. There were five treatments of irrigation consisting four levels of irrigation viz. 1.0, 0.85, 0.70, and 0.55 volume applied through drip and 1.0 volume of irrigation through surface (flood irrigation) method. The three sub treatments of with and without mulch of 25 micron LLDPE black polythene film (mulch) and organic mulch "Kheep" (Dry Kheep was used @ 0.5 kg /running meter). The experiment was laid out in split plot design and was replicated four times.

The daily irrigation was applied as per plant requirement and calculated on the basis of class 'A' pan evaporation data. The volume of water required was calculated by multiplying the pan evaporation with the crop coefficient at different crop stages, pan coefficient, the plant to plant spacing, the row to row spacing and per cent wetted area (Rana et al., 2004). The calculated water on the basis of pan evaporation was applied as per treatment. In surface irrigation, measured quantity of water required by plant per day was applied.

Results and discussion

The length of fruit was significantly influenced by application of different volume of water in both years (2006 and 2007). The maximum length of fruit was recorded under the treatment drip 0.70 volume which was significantly higher than rest of the treatment. The higher number of fruits per plant was recorded with drip 0.70 water volume treatment i.e. 10.63 and 10.12 while the lowest i.e. 8.74 and 8.67 with the surface irrigation treatment in both the years of observation (2006 and 2007), respectively. The application of water volume 0.70 significantly influenced the average weight of fruit (448 and 443 g) and yield of fruit (401.6 and 391.2 q) than the surface irrigation in 2006 and 2007, respectively. To achieve the maximum yield under 0.70 water volume, the average water quantity 2.26 and

2.14 liter /day/plant was applied, however, it was applied 3.23 and 3.06 liter in surface irrigation treatment in 2006 and 2007, respectively. The probable reason is that the water volume 0.70 seems to be optimum water quantity which is required by plant, however more water volume increase the vegetative growth of plant which inhibited the exposure of sunlight, which is essential for flowering and fruiting (Singh and Gangwan, 1972). Different mulches viz. no mulch, polythene mulch and organic mulch showed significantly variation on yield and yield attributing characters in the study. It is evidence from the table -1 that the soil temperature was influenced by the different mulches and it was minimum (30.02, 29.92 °C) under organic mulch than no mulch (35.02, 35.27 °C) however, it was maximum under polythene mulch (40.29, 40.56 °C) in both years (2006 and 2007), respectively. Similarly, average number of fruit per plant and average weight of fruit were increased under organic mulch; however, black polythene mulch could not influence both the parameters. The highest fruit yield (406.8 and 395.6 q/h) was obtained with organic mulch whereas no mulch recorded only 365.6 and 358.4 q/ha. in 2006 and 2007, respectively. Because, the organic mulch developed of suitable soil moisture regime favorable for efficient nutrient uptake and also increase microbial population resulting in better biological growth and higher harvest (Mangal, 1990 and yadav, 2007). The organic mulch maintains the both temperature and moisture in soil which is essential for better root development and other physiological activities of the plant. On the other side, a great reduction was observed in yield under black polythene mulch in comparison to control. It may be attributed to increase in temperature beyond the optimum limit under black polythene which inhibits the physiological activity of plant

It is evident from the study that the significantly higher No. of fruit (11.85 and 10.75) and maximum yield (493.4 and 476.0 q/h) were recorded with application of 0.70 volume of water with organic mulch in both years 2006 and 2007, respectively.

Treatments	Soil temperature at 40°C (upto 30 cm depth)		Length of fruit (cm)		Average No. of fruits per plant		Average weight of fruit (gm)		Yield (q/ha)		Average water applied (Lt/day/plant)	
	2006	2007	2006	2007	2006	2007	2006	2007	2006	2007	2006	2007
Surface irrigation	35.67	35.77	31.79	31.45	8.74	8.67	378	373	274.9	271.2	3.23	3.06
Drip 1.0 water volume	34.56	34.62	33.81	32.65	9.79	9.43	399	391	325.7	325.6	3.23	3.06
Drip 0.85 water volume	34.92	35.12	34.69	33.65	10.03	9.78	432	408	363.9	354.4	2.74	2.60
Drip 0.70 water volume	35.18	35.27	36.41	33.68	10.63	10.12	448	433	401.6	391.2	2.26	2.14
Drip 0.55 water volume	35.23	35.42	34.55	33.42	10.45	9.80	443	405	382.0	371.5	1.78	1.68
S.Em. ±	0.31	0.28	0.37	0.39	0.08	0.056	3.18	3.68	2.63	10.55	-	-
CD at 5%	NS	NS	1.14	1.29	0.25	0.173	9.80	11.36	8.11	32.51	-	-
No mulch	35.02	35.27	36.18	35.46	9.93	9.54	439	413	365.6	358.4	2.65	2.51
Polythene mulch	40.29	40.56	28.64	28.26	9.21	9.21	361	358	276.5	273.2	2.65	2.51
Organic mulch	30.02	29.92	37.94	36.39	10.65	9.94	454	435	406.8	395.6	2.65	2.51
S.Em. ±	0.75	0.82	0.21	0.28	0.04	0.038	2.20	2.30	2.06	6.40	-	-
CD at 5%	2.50	2.67	0.61	0.85	0.13	0.11	6.35	6.65	5.97	18.51	-	-

Table 2. Interaction effect of water volume and mulches on the yield bottle gourd (year 2007)

Treatments	Yield (q/ha)						Number of fruit per plant					
	Without mulch		Polythene mulch		Organic mulch		Without mulch		Polythene mulch		Organic mulch	
	2006	2007	2006	2007	2006	2007	2006	2007	2006	2007	2006	2007
Surface irrigation	284.0	280.7	246.1	240.5	294.8	292.7	8.85	8.78	8.43	8.38	8.95	8.85
Drip 1.0 water volume	334.8	328.5	277.9	295.0	364.4	351.5	9.75	9.25	4.26	9.21	10.36	9.85
Drip 0.85 water volume	396.5	385.2	281.3	271.0	414.1	406.5	10.31	9.75	9.32	9.45	11.46	10.14
Drip 0.70 water volume	420.7	414.2	290.6	284.2	493.4	476.0	10.51	9.12	9.54	9.51	11.85	10.75
Drip 0.55 water volume	392	385.2	286.5	277.5	467.4	452.5	10.22	9.79	9.48	280.7	11.65	10.10
S.E.m. ±	4.62	14.33	-	-	-	-	0.1006	0.0856	-	-	-	-
CD at 5%	13.35	41.39	-	-	-	-	0.2906	0.2474	-	-	-	-

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Role of Trainings to rural masses in value addition of arid fruits and vegetables

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Horticultural produces grown in arid region are of explicit quality with great nutritional, medicinal, organoleptic and economic importance. Some of the arid vegetables like *kachri*, *mateera*, clusterbean etc. and fruits such as *ker*, *khejri*, *ber*, *lasora*, *guarpatha* and *aonla*, etc. on barren lands are available to the rural masses to provide food and nutrition security. Most of the arid food resources are available in plenty during a particular season, but all cannot be utilized to the fullest extent due to many reasons. Thus people residing in arid areas hardly get on considerable advantage from the abundantly available resources. Rural masses consume these food resources as such available to them in the form of simple vegetable or by simply in the form of dehydrated product or fruits are consumed in raw form. However, there is much scope in evolving the techniques of value addition and providing market to such underutilized commodities for more net returns.

Various standardized techniques are practiced commercially for processing of arid fruits and vegetables (Table 1).

Table 1. Some potential value added processed products of arid horticultural crops

S. No.	Crops	Value added products
1	Ber	Ber candy, preserve, squash, RTS, Jam, chutney, pickle, powder
2	Aonla	Preserve, candy, shreds, powder, chyawanprash, pickle
3	Ker	Powder, Pickle
4	Aloe	Jam, squash, laddu, biscuit, pickle
5	Khejri	Sangri, pickle, biscuits, laddu
6	Mateera	Squash, RTS
7	Watermelon	Squash, RTS
8	Kachri	Dried vegetable, pickles, chutney
9	Clusterbean	Dried pickle

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Negligible quantities of value added products from arid fruits and vegetables compared to their production are available in the market. Thus rural masses can choose this enterprise for their employment and income generation. It requires proper technical knowledge and skill for preparing these value added products. For technical guidance proper trainings are required. Trainings can be helpful in creating awareness among the rural masses as well as can provide technical knowledge and skill in specific aspects. Krishi Vigyan Kendra, Bikaner is one such Institutes which provides various types of trainings to the rural masses including farmers, farm women and rural youth. Krishi Vigyan Kendra organizes various types of trainings as vocational trainings, on campus trainings and off-campus trainings (Table 2). During last five years 10 off campus, 6 on-campus and 1 vocational training were organized by Krishi Vigyan Kendra on value addition. Lecture cum-interactive demonstration technique were followed in the trainings in which participants themselves were involved in preparing value added products which instilled lot of confidence in them.

Table 2. Type of No. of Trainings on preparation of value added products organized by KVK, Bikaner during 2003 to 2007

S. No.	Type of trainings	Duration	No. of trainings
1	Off-campus	1 day	10
2	On-campus	2-3 days	6
3	Vocational	36 days	1
(under Rajasthan Mission of Livelihood, Jaipur)			

One 36 days vocational training on fruit and vegetable preservation was organized under the sponsorship of Rajasthan Mission of Livelihood (RMOL) Jaipur. This training also included other aspects related to marketing, labeling, packaging, Banking, etc. to make them confident in establishing their own small scale enterprise for self-employment.

Other extension activities like exhibitions and distribution of folders/leaflets help in motivating masses for utilizing the value added products. Five such exhibitions on value addition of arid fruits and vegetables were organized in which recipes prepared by trainees were displayed and awards were distributed to trainees whose recipes were adjudged the best. Aonla value added products by way of these above mentioned extension techniques have been popular from the last 4-5 years in Bikaner district (Table 3) and have motivated the fruit growers and unemployed rural women for income generation.

Table 3. Extension activities carried out on value addition of arid horticultural crops

S. No.	Type of activity	Title	Name of Author(s)
	Folders/Pamphlets	आंवला के विभिन्न उत्पाद	डॉ. नीना सरीन, डॉ. इन्द्रमोहन वर्मा, डॉ. अतुल चन्द्र एवं श्री अमरलाल गौड़
-do-		फल सब्जी परिरक्षण - एक व्यवसाय	डॉ. नीना सरीन, डॉ. इन्द्रमोहन वर्मा एवं डॉ. अतुल चन्द्र
-do-		विभिन्न प्रकार के अचार	डॉ. नीना सरीन
Exhibitions		Five exhibitions conducted	

After receiving the trainings 80 per cent of the participants started preparing some of the selected products like aonla, pickle, preserve, chutney, etc. at the household level. Three participants started their own units espe-

cially on Aonla Produces and are earning a good amount of money. Mrs. Prem Kumari Tanwar has established her own small scale unit on Aonla products (aonla juice, preserve of candy) and developed a good market in Bikaner and outside state also. Shri Amar Singh Sangwa has expertised in preparing Chywanprash and is earning good amount of money. Mr. Gopal Rathi has prepared dried aonla products.

Thus, considering the nutritional and medicinal importance of arid fruits and vegetables, general public has been attracted towards the value added products and have started consuming these products which in turn can give the economic benefit to the rural masses in general and farm women's and producers of fruits and vegetables in particular.

Most of the arid food resources are available in plenty during a particular season but all can not be utilized to the fullest extent due to many reasons. Thus people residing in arid areas hardly get on considerable advantage from the abundantly available resources. There is much scope in evolving the techniques of value addition and providing market to such underutilized commodities for more net returns. It requires proper technical knowledge and skill for preparing these value added products.

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Effect of gamma irradiation on seed germination and survival of seedlings of pomegranate (*Punica granatum* L.)

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Pomegranate (*Punica granatum* L.) is the important crop of arid and semi-arid regions of India. Maharashtra is the leading state for area and production of pomegranate followed by Karnataka and Andhra Pradesh. At present, Maharashtra occupies about 92,000 ha area out of 1.3 lakh ha area available under pomegranate in India. In pomegranate, genetic base is very narrow. Therefore, creation of variability is utmost important need for crop improvement programme. Attempts have been made earlier to induce mutation in pomegranate by treating seeds with gamma rays (5-20 kR) and various forms were obtained with desirable traits. However, the dwarf mutation forms were most commonly observed in pomegranate (Akhund-zade-IM, 1979 and Levin, 1990). Gamma irradiation as a breeding tool to create variability in both horticulture and field crops was reported by several workers (Haskins and Chapman, 1956; Mikaelson, 1968 and Pathak, 2003). Several released varieties of field and fruit crops have been enlisted which were developed through gamma irradiation (Anon., 1981). Considering the significance of gamma irradiation in crop improvement, the present study was under taken and response of gamma irradiation on seed germination and subsequent survival of seedlings of pomegranate was studied.

The present investigation was carried out at the National Research Centre on Pomegranate, Solapur, Maharashtra during 2006-07. The seeds of pomegranate cv. Ganesh was extracted and dried in shade during January 2007. The dried seeds were treated with gamma radiation at Bhabha Atomic Research Centre, Trombay, Mumbai. In all, 11 gamma radiation treatments viz., 0, 3, 6, 9, 12, 15, 18, 21, 24, 27, 30 kR with four replications were taken for the present study. In each replication, 50 seeds were sown in polythene bags containing sand, soil and FYM in 1:1:1 ratio during first week of May 2007. The experiment was laid out in randomized block design. Standard nursery practices were followed during the experimentation. The seed germination was recorded on 10, 14, 18, 22 and 26 days after sowing (DAS) and expressed in terms of per cent germination. Survival of germinated seedlings was counted on 70 DAS and expressed as per cent survival. The data were analyzed statistically and used for interpretation of the results.

Seed germination

The seed germination was significantly influenced by gamma irradiation treatments at different germination stages (Table 1). In general, the germination started from 8 days after sowing (DAS) and continued till 26 DAS in different treatments. On 10 DAS, seed germination ranged from 4.0 to 29.5 per cent. The seed germination was higher at control, 3 kR and 6 kR and their values were at par to each other. Beyond 6 kR treatments, the seed germination was decreased and it was noted minimum (4.0 %) at 30 kR indicating that higher doses (> 6 kR) of gamma rays had negative effect on germination.

On 14 DAS, the germination ranged from 11.5 to 55.0 per cent. Control, 3kR and 6kR showed higher germination which ranged from 51.5 to 55.0 per cent, but these treatments were at par to each other. Beyond 6 kR, the germination per cent gone below 50.0 per cent and in general, the germination decreased gradually with increasing doses of gamma irradiation and thus, the minimum germination was recorded at 30 kR (11.5%). The same trend was noted at 18 DAS and 22 DAS also and their germination per cent ranged from 19.5 - 60.5 and 23.0 - 63.5, respectively.

On 26 DAS, almost all the germinable seeds have germinated and after that, no further germination was noted. The germination per cent ranged from 23.0 and 67.5 in different treatments. Although, the maximum seed germination (67.5 %) was recorded with 6kR but control, 3 kR and 6 kR treatments were at par. Beyond 6 kR treatments, the seed germination, in general, was in decreasing trend and recorded minimum (23.0 %) at 27 kR which was at par with 24 kR and 30 kR. Interestingly, 9 kR and 12 kR treatments were at par to each other and their values ranged from 41.5 and 47.0 per cent showing below 50.0 % seed germination. Now, it is clear from the data that LD 50 for cv. Ganesh would be 9 kR or 12 kR. However, irradiation treatment beyond 6 kR was found to be lethal as they showed inhibitory effect on seed germination. The decrease in germination percentage of seeds might be attributed to the possible chromosomal aberrations caused by the mutagens (Gordon and Weber, 1955). According to Evans (1965), the reduction in germination of irradiated seeds is a cumulative expression of at least three different types of effects occurring in the meristematic cells that is cytologically identifiable. Earlier reports indicate that seed

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treatments with 5-20 kR gamma rays in pomegranate induced mutation and produced different desirable forms including dwarf types (Akhund-zade-IM, 1979; Levin, 1990). Our study (not included) also indicated that in irradiated seedling population some dwarf forms are there and they will be reported separately.

Survival of seedlings

Regarding the survival of seedlings on 70 DAS, the survival ranged from 8.9 to 95.4 per cent and the treatments differed significantly (Table 1). The survival of seedlings did not differ significantly among control to 15 kR treatments and their values ranged from 92.1- 95.4 per cent. Subsequently the survival percentage decreased after 15kR with increasing doses of gamma radiation and least values recorded at 27 kR and 30 kR (8.9 to 10.4 %). The lethal dose for survival of 50 per cent seedlings was found to be close to 24 kR. Beyond 24 kR, the gamma irradiation was found to have deleterious effect on the survival of seedlings. The reduction in survival per cent at higher doses of gamma radiation may be attributed to cell death and higher rate of ionization in the nuclei. The drastic decrease in survival percentage beyond 18 kR may be due to physiological imbalance and damages caused at the molecular level, which results in chromosomal aberrations causing considerable cytological changes. This is in line with the findings of Vishwanathan *et al.*, (1992) in their observation on the effect of gamma irradiation in *Kaempferia*. The reduction in survival of irradiated seedlings might be attributed to auxin destruction in the juvenile seedlings (Skoog, 1935) and failure of the assimilatory mechanisms in the later stage (Quastler and Baer, 1950).

From the study, it is concluded that LD 50 for cv. Ganesh found to be 9 kR or 12 kR. However, up to 15 kR doses, there was no deleterious effect on survival of pomegranate seedlings. Among different irradiation doses tested, 27 kR and 30 kR were most deleterious and caused very poor survival of seedlings.

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Table 1. Effect of gamma irradiation on seed germination and seedling survival of pomegranate cv. Ganesh

Treatment	Germination (%)					Survival (%) at 70 DAS
	10 DAS	14 DAS	18 DAS	22 DAS	26 DAS	
Control	29.5	51.5	55.0	58.5	61.5	95.4
3 kR	29.5	55.0	60.0	63.5	64.5	93.3
6 kR	24.0	53.5	60.5	62.5	67.5	95.1
9 kR	16.0	31.0	41.5	46.0	47.0	92.1
12 kR	18.0	32.0	38.5	40.0	41.5	92.4
15 kR	12.0	21.3	26.0	29.0	33.0	93.5
18 kR	7.50	15.5	19.0	21.5	22.5	85.1
21 kR	8.0	19.5	29.5	33.5	35.0	70.3
24 kR	9.0	18.0	24.0	26.5	28.0	54.2
27 kR	6.0	16.0	19.5	25.0	23.0	8.9
30 kR	4.0	11.5	19.0	23.0	23.5	10.4
CD (0.05)	9.06	6.46	7.45	8.67	9.06	8.72

Estimation of genetic parameters, character associations and path analysis for fruit yield and its components in Ber (*Ziziphus mauritiana* Lamarck)

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Arid region in India is spread over 38.70 million ha covers about 12 per cent of the total area of the country. The tracts of hot arid region are found in the states of Rajasthan (19.6 m ha) followed by Gujarat (6.2 m ha), Andhra Pradesh (2.2 m ha), Punjab (1.5 m ha), Haryana (1.3 m ha), Karnataka (0.8 m ha) and Maharashtra (0.1 m ha). Farming being a difficult task in the arid tropics, the agro-climate of such regions favours the cultivation of certain fruit crops. Indian jujube (*Ziziphus mauritiana* Lamk.) commonly known as *Ber* belonging to family Rhamnaceae, is indigenous to India and is one of the important fruit trees that can be successfully cultivated in the hot arid regions of India. The fruits of Indian jujube are rich in vitamin C, A and B complex. In Punjab it is cultivated on 2,894 ha having annual production 43,410 mt (Anon. 2006). Out of more than 90 cultivars only 11 are commonly cultivated commercially in different agro climatic regions of India (Morton 2005).

The choice of a suitable cultivar is of paramount importance for its success. The breeding strategy for genetic improvement of fruit yield and its components depends upon the genetic diversity for different traits. The assessment of genetic parameters like genotypic coefficient of variability (GCV), phenotypic coefficient of variability (PCV), heritability (h^2) and genetic advance is a pre-requisite for making effective selection and improvement in the base population.

Path analysis based on phenotypic correlation coefficients further unravels the contributions of different traits towards fruit yield. Presently very little information is available on this crop regarding genetic parameters to be helpful for the effective selection leading to an ideal genotype having high fruit yield. Thus the present investigation was under taken to assess the nature and magnitude of genetic parameters and their utilization for

the development of superior genotypes of Indian jujube suitable for semi arid regions of Punjab.

The experimental material comprising of 9 different cultivars viz., *Gola*, *Muria*, *Murhera*, *Sandhura*, *Narnaul*, *Katha phal*, *Umran*, *Seb*, *Najuk*, *Chuhara* and *Walaiti* collected from different parts of India during 1995-96 was accommodated in randomized block design with four replications at PAU, Regional Research Station, Bathinda, Punjab. The average rainfall at the experimental site was 400 mm. The annual maximum temperature was 31.45 °C and annual minimum temperature was 16.85 °C. The soil was sandy loam and characterized with pH (8.31), organic carbon (0.32%), electrical conductivity (0.24 dS/m), available N (212 Kg/ha), available P (21.5 Kg/ha) and available K (357.0 Kg/ha). In each replication 3 plants of each cultivar were grown at geometrical spacing of 7.5 x 7.5 metres. Data were recorded on fruit yield per plant (kg), fruit weight (g), fruit length (cm), fruit breadth (cm), stone weight (g), total soluble solids (TSS), acidity (%), pulp percentage and pulp: stone ratio for five years 2002-03, 2003-04, 2004-05, 2005-06 and 2006-07. The genotypic and phenotypic coefficient of variation and heritability (in broadsense) were computed as suggested by Singh and Choudhary (1979), while genetic advances were estimated as per the procedure of Johnson *et al.* (1955). The path analysis was carried out following Dewey and Lu (1959).

The analysis of variance showed significant differences among the cultivars for all the nine traits. The high estimates (>15%) of GCV recorded for stone weight, fruit weight, pulp: stone ratio, fruit yield per plant, total soluble salts, and fruit length, indicated the presence of adequate genetic variation among the genotypes and suitability of these attributes for further improvement by selection. The heritability estimates were high for all the traits (Table 1). Nanohar *et al.* (1986) also reported that the fruit weight, yield per plant and pulp:stone ratio showed high GCV. The heritability estimates were high for all the traits (Table 1). Nanohar *et al.* (1986) and Bisla and Daulta (1986) also reported moderate to high estimates of heritability for various characters along with high genetic

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gains in this crop. As characters like stone weight, fruit weight, pulp stone ratio, fruit yield per plant and total soluble salts showed high heritability estimates coupled with high genetic advances, it can be inferred that these traits are under the control of additive gene action and phenotypic selection for their improvement will be effective. Kaushik *et al.* (2004), Saran *et al.* (2007) and Tomar and Singh (1987) also reported considerable range of genetic variability for these traits in their germplasm.

The phenotypic correlation coefficients between different characters are depicted in table 2. The estimates of correlation coefficients showed significant positive association of fruit yield per plant with pulp percentage (0.5467) and pulp: stone ratio (0.5329) while negative association with stone weight (-0.5166) and total soluble salts (-0.3567). Among the other components fruit weight was positively correlated with all other traits except stone weight and acidity. Fruit length as well as breadth showed positive association with total soluble salts while negative association with acidity and stone weight. Pareek and Dhaka (2003) also observed that fruit length, fruit breadth, fruit weight and pulp weight had significant positive association with fruit yield. Pulp percentage and pulp: stone had negative association with total soluble salts, acidity and stone weight. Significant association among fruit yield components indicated that correlated response to selection for these traits will result in ultimate simultaneous improvement of fruit yield. Heritability estimates were higher for component traits as compared to fruit yield per plant. Also the GCV values were closer to PCV values for components than for fruit yield. So it can be concluded that components are less susceptible to environmental fluctuations than yield *per se* and are thus relatively more amenable to improvement.

Path analysis carried out to estimate the direct and indirect contributions of various component traits for recommending a reliable selection criterion revealed that fruit weight (0.6381) followed by pulp percentage (0.5050) and total soluble salts (0.4997) had high direct effects on fruit yield (Table 3). Fruit weight also showed indirect contribution via fruit length (0.3378), fruit breadth (0.4463), pulp percentage (0.4284) and pulp: stone ratio (0.4115) revealing that fruit weight is the major contributor towards

fruit yield per plant. Fruit length and fruit breadth also contributed via total soluble salts and pulp percentage. Highest direct effect of fruit length was also reported by Pareek and Dhaka (2003). It is concluded that during selection due weightage should be paid to fruit weight, total soluble salts and pulp percentage for the development of suitable cultivars of *Indian jujube* for semi arid region of Punjab.

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Table 1. Estimates of genetic parameters for fruit yield and its components in Ber

Characters	GCV	PCV	Heritability (h ²)	Genetic advance	GA (%)	Mean	CV (%)
Fruit yield per plant (kg)	19.88	21.18	88.08	19.01	38.44	49.45	7.31
Fruit weight (g)	22.82	23.45	94.66	9.26	45.73	20.25	5.42
Fruit length (cm)	17.19	17.38	97.87	1.51	35.03	4.31	2.54
Fruit breadth (cm)	11.12	11.18	99.02	0.68	22.80	2.96	1.10
Total soluble salts (%)	18.28	18.56	97.01	0.43	37.08	1.17	3.21
Acidity (%)	10.95	11.01	98.87	3.70	22.42	16.51	1.17
Stone weight (g)	36.64	36.93	98.45	0.32	74.89	0.42	4.59
Pulp (%)	1.32	1.33	97.75	2.52	2.68	93.99	0.20
Pulp stone ratio	22.69	24.86	83.31	7.17	42.66	16.80	10.15

Table 2. Phenotypic correlation coefficients of seed yield and component characters in Ber

Characters	Fruit yield per plant (kg)	Fruit weight (g)	Fruit length (cm)	Fruit breadth (cm)	Total Soluble Salts (%)	Acidity (%)	Stone weight (g)	Pulp (%)	Pulp stone ratio
Fruit yield per plant (kg)	1.0000								
Fruit weight (g)	0.2577	1.0000							
Fruit length (cm)	0.0761	0.5294**	1.0000						
Fruit breadth (cm)	-0.0687	0.6995**	0.0419	1.0000					
Total Soluble Salts (%)	-0.3567*	0.4357**	0.3678*	0.6134**	1.0000				
Acidity (%)	-0.1454	0.4871**	0.4917**	0.5068**	-0.2527	1.0000			
Stone weight (g)	0.5166**	-0.3355	-0.4012*	0.1410	0.2091	0.0875	1.0000		
Pulp (%)	0.5467**	0.6713**	0.3064	0.2503	0.3442*	0.3647*	0.5940**	1.0000	
Pulp stone ratio	0.5329**	0.6450**	0.2412	0.1433	0.3861*	-0.2217	0.4958**	0.9336	1.0000

*Significant 5% level of probability

**Significant 1% level of probability

Table 3. Path analysis coefficients of fruit yield versus component characters in Ber.

Characters	Fruit weight (g)	Fruit length (cm)	Fruit breadth (cm)	Total soluble salts (%)	Acidity (%)	Stone weight (g)	Pulp (%)	Pulp stone ratio	PCC* with fruit yield
Fruit weight (g)	0.6381	-0.4883	-0.7980	0.2177	0.2594	0.0907	0.3390	-0.0009	0.2577
Fruit length (cm)	0.3378	-0.9225	-0.0478	0.1838	0.2619	0.1084	0.1547	-0.0003	0.0761
Fruit breadth (cm)	0.4463	-0.0386	-1.1409	0.3065	0.2699	-0.0381	0.1264	-0.0002	-0.0687
Total Soluble Salts (%)	0.2780	-0.3393	-0.6998	0.4997	0.1346	-0.0565	-0.1738	0.0005	-0.3567
Acidity (%)	-0.3108	0.4536	0.5782	-0.1263	-0.5326	-0.0236	-0.1842	0.0003	-0.1454
Stone weight (g)	-0.2141	0.3701	-0.1609	0.1045	-0.0466	-0.2702	-0.3000	0.0007	-0.5166
Pulp (%)	0.4284	-0.2827	-0.2855	-0.1720	0.1942	0.1605	0.5050	-0.0012	0.5467
Pulp stone ratio	0.4115	-0.2225	-0.1635	-0.1929	0.1181	0.1340	0.4714	-0.0233	0.5329

Bold figures are direct effects
Residual effect: 0.5132

*PCC stands for phenotypic correlation coefficient

Effect of antioxidant and mustard oil emulsion on post harvest shelf life of fresh fruits of date palm cv. Barhee

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Date palm (*Phoenix dactylifera* L.) is a highly nutritious fruit crop. It is usually eaten as fresh (fruits harvested at khalal stage) and fully ripened fruit (harvested at pind or tamer stage). Date is highly nutritious fruit. Besides carbohydrate and protein, date fruit provides small amount of copper, magnesium, chlorine, sulphur, Vit A₁, B₁ and B₂. The following contents are an average for all date varieties at khalal stage: moisture 59.2%, nitrogen 1.2%, fat 0.4%, carbohydrate 33.8%, energy 144 calories/kg, calcium 22 mg/100g, and phosphorus 38 mg/100gm.

Its origin is not known exactly but is an ancient fruit indigenous to countries near to Persian Gulf. Till date in India the commercial plantations are restricted only to Kachchh district of Gujarat. Due to the climatic conditions of the region fruits do not reach to pind or tamer stage and hence growers are compelled to harvest the fruits at khalal stage (as fresh fruits). Earlier there was a little market of these fruits restricted to Kachchh and some parts of Saurashtra region in Gujarat. But now-a-days market of fresh fruits has spread to far southern states viz. Maharashtra, Karnataka, Tamilnadu and Kerala.

Like other horticultural commodities date fruits are also perishable. With the increase in demand of fresh fruits to far away places, it is challenging for the growers and traders to keep their fruits in fresh condition till they reaches to consumer.

Antioxidants have proved to improve the post harvest shelf life of various fruits (Jayachandran *et al.*, and Renhua Huang *et al.* 2007). Presence of antioxidant content in fruit and vegetable species itself has been found to enhance their keeping quality (Claire Hebert and Louis Gauthier, 2007). Several antioxidants are easily available. Out of hence, ascorbic acid considered as safe and on the other hand it adds nutritive value also.

Use of edible oils to extend the shelf life of horticultural produce is an old practice. Several workers

have used mustard oil emulsion to extend the post harvest shelf life (Verma *et al.*, 1999, Verma and Dashora, 2000).

In the present study, these two approaches were used alone and in combination to see their effect on post harvest shelf life of fresh date fruits of cv. Barhee.

Mature Barhee dates at khalal stage were harvested from the same tree from 11th block of farm of Date palm Research Station, Mundra – Kachchh. The orchard is under standard agricultural practices with proper pest management. Soil of orchard is sandy to sandy loam. Fruits were brought to laboratory for treatment immediately after harvesting. Fruits were thoroughly cleaned with a piece of cloth. After that they were subjected to different treatments. Fruits were dipped in 0.1 and 0.2 per cent ascorbic acid solutions separately. For mustard oil emulsion treatment first oil was added to water (for 4 % solution 40 ml mustard oil in 1 litre water.) and then emulsion was made using teepol so that oil could mix thoroughly in water. In case of combination of treatment fruits were treated first with ascorbic acid and then dipped in oil emulsion.

Fruits were given dipping treatment of 20 seconds and 5 seconds for ascorbic acid and oil emulsion respectively. An absolute control was also maintained. After this, total six treatment combinations viz. (i) control, (ii) mustard oil emulsion @ 4%, (iii) ascorbic acid @ 0.1%, (iv) ascorbic acid @ 0.1% + mustard oil emulsion 4%, (v) ascorbic acid @ 0.2%, (vi) Ascorbic acid @ 0.2% + mustard oil emulsion 4% were tried. Each treatment was replicated thrice. After proper treatment fruits were kept in perforated polyethylene bags of 100 gauge. Each bag was having 3 strands of date palm. All the treatments were kept in ambient condition. Initial observations were recorded before subjecting the fruits to treatments. Later observations were recorded on 4th, 8th and 12th day of experiment. Treatments were evaluated for PLW (%), rotting (%), marketability (%), fruit weight (g), seed weight (g), pulp: stone ratio and TSS (%). PLW was measured using the following formula:

$$PLW\% = \frac{\text{Initial weight} - \text{Final weight}}{\text{Initial weight}} \times 100$$

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Rotting and marketability were recorded by visual observation. Fruit and seed weight were recorded by simply weighing the fruit. TSS was recorded by hand refractometer. Experiment was laid out in factorial in completely randomized design using the technique of Cochran and Cox (1950).

Results are presented in Tables 1 and 2. Perusal of data indicated that with the use of 4 per cent mustard oil emulsion, per cent PLW, rotting per cent and TSS increased while marketability, fruit weight and pulp: stone ratio decreased significantly over without oil. So it is clear that ripening process enhanced by the use of oil emulsion instead of retarding it.

After this analysis, the comparison of main effect of ascorbic acid will not give the right idea, hence in the present study, comparison among ascorbic acid treatment alone i.e. without oil was made to know the effect of ascorbic acid. Minimum PLW of 2.53, 7.46 and 10.95% was recorded in fruits treated with 0.2% ascorbic acid while maximum of 3.60, 9.60 and 17.57% in control on 4th, 8th and 12th day respectively. Same trend was also recorded in rotting and marketability also where minimum rotting i.e. 4.05, 20.73 and 26.19% was found in 0.2% ascorbic acid treated fruits which was significantly at par with 0.1% ascorbic acid while maximum rotting of 10.60, 28.79 and 33.65% was recorded in control on 4th, 8th and 12th day

respectively. Marketability per cent was also non significantly affected by the concentration of ascorbic acid, though maximum marketability 85.91, 70.11 and 64.50% was observed in ascorbic acid @ 0.2 per cent and minimum in control (80.21, 61.85 and 56.93%) on 4th, 8th and 12th day of storage, respectively.

Perusal of Table 2 revealed that maximum fruit weight (11.78, 10.00 and 10.51 g on 4th, 8th and 12th day, respectively) was recorded in ascorbic acid 0.2 per cent treated fruits. Effect on pulp: stone ratio and TSS was found to be non significant. Better effect of ascorbic acid to enhance shelf life is due their role in protecting the tissue against stress and participates to disease resistance also (Claire and Gauthier, 2007). In the cell or tissue system first they get digested and then reduce the respiration process. Present study provides evidence that post harvest shelf life of cv. Barhee can be increased by using ascorbic acid, which is well supported by Farag (1998) in date palm, Jayachandran *et al.*, (2007) in guava and Mohammed and Wickham (2005) in pineapple.

Oil emulsion enhanced the ripening process which reduces the shelf life instead of improving it. Though the several workers have been found to increase the shelf life of other fruits (Verma *et al.*, 1999 and Verma and Dashora, 2000). But result of the present study were not in line them and oil emulsion was not able to prevent

Table 1. Effect of ascorbic acid and mustard oil emulsion on PLW, rotting % and marketability %.

	PLW								
	4 th day			8 th day			12 th day		
	Without oil	With oil	Mean	Without oil	With oil	Mean	Without oil	With oil	Mean
Initial value = 0									
Without Asc. acid	3.60	3.66	3.63	9.60	9.50	9.55	17.57	23.43	20.50
Asc. Acid (0.1%)	3.03	4.26	3.65	8.16	9.00	8.58	11.99	20.58	16.29
Asc. Acid (0.2%)	2.53	5.40	3.96	7.46	10.76	9.11	10.95	20.94	15.95
Mean	3.05	4.44		8.41	9.75		13.51	21.65	
	SEm+	CD (5%)		SEm+	CD (5%)		SEm+	CD (5%)	
Ascorbic acid (A)	0.030	0.094		0.105	0.325		0.902	2.778	
Oil emulsion(B)	0.025	0.077		0.086	0.265		0.736	2.269	
A X B	0.043	0.133		0.149	0.459		1.275	NS	
Rotting (%)									
Initial value = 0									
Without Asc. acid	10.60	21.00	15.80	28.79	51.25	40.02	33.65	70.90	52.28
Asc. Acid (0.1%)	4.05	21.18	12.61	23.17	60.13	41.65	28.51	74.22	51.36
Asc. Acid (0.2%)	4.05	22.98	13.51	20.73	57.80	39.26	26.19	75.22	50.70
Mean	6.23	21.72		24.23	56.39		29.45	73.45	
	SEm+	CD (5%)		SEm+	CD (5%)		SEm+	CD (5%)	
Ascorbic acid (A)	1.025	NS		0.524	1.614		1.333	NS	
Oil emulsion(B)	0.837	2.578		0.428	1.318		1.088	3.353	
A X B	1.449	4.465		0.741	2.283		1.885	5.807	
Marketability (%)									
Initial value = 100									
Without Asc. acid	80.21	69.83	75.02	61.85	39.29	50.57	56.93	20.03	38.48
Asc. Acid (0.1%)	85.91	69.65	77.78	67.59	30.48	49.03	62.14	16.81	39.47
Asc. Acid (0.2%)	85.91	67.78	76.84	70.11	32.79	51.45	64.50	15.86	40.18
Mean	84.01	69.09		66.51	34.19		61.19	17.57	
	SEm+	CD (5%)		SEm+	CD (5%)		SEm+	CD (5%)	
Ascorbic acid (A)	0.918	NS		0.533	1.644		1.295	NS	
Oil emulsion(B)	0.749	2.309		0.436	1.342		1.057	3.257	
A X B	1.298	3.999		0.754	2.325		1.831	5.642	

Table 2. Effect of ascorbic acid and mustard oil emulsion on fruit weight (g), seed weight (g), pulp: stone ratio and TSS (%).

Fruit weight (g)	Initial value = 12.19 4 th day			8 th day			12 th day		
	Without oil	With oil	Mean	Without oil	With oil	Mean	Without oil	With oil	Mean
Without Asc. acid	11.09	10.18	11.09	9.70	9.63	9.66	10.08	10.39	10.23
Asc. Acid (0.1%)	11.03	10.43	10.43	10.00	8.98	9.49	9.40	8.38	8.89
Asc. Acid (0.2%)	11.78	11.61	11.20	10.00	9.04	9.52	10.57	7.93	9.22
Mean	11.27	10.74			9.92		9.99	8.90	
	SEm±	CD (5%)		SEm±	CD (5%)		SEm±	CD (5%)	
Ascorbic acid (A)	0.149	NS		0.071	NS		0.291	0.895	
Oil emulsion(B)	0.122	0.375		0.058	0.178		0.237	0.731	
A X B	0.211	0.650		0.100	0.309		0.411	1.266	
Seed weight (g)									
	Initial value = 1.04								
Without Asc. acid	1.25	1.34	1.30	1.11	1.11	1.11	1.18	1.25	1.21
Asc. Acid (0.1%)	1.18	1.14	1.16	1.12	0.99	1.05	1.05	1.12	1.08
Asc. Acid (0.2%)	1.17	1.12	1.14	1.18	1.11	1.14	1.10	1.15	1.12
Mean	1.20	1.20		1.14	1.07		1.11	1.17	
	SEm±	CD (5%)		SEm±	CD (5%)		SEm±	CD (5%)	
Ascorbic acid (A)	0.029	0.091		0.013	0.041		0.041	NS	
Oil emulsion(B)	0.024	NS		0.011	0.034		0.033	NS	
A X B	0.042	NS		0.019	0.058		0.057	NS	
Pulp: Stone ratio									
	Initial value = 10.72								
Without Asc. acid	7.80	7.31	7.55	7.69	7.67	7.68	7.54	7.28	7.41
Asc. Acid (0.1%)	8.32	8.14	8.23	7.92	8.04	7.98	7.92	6.50	7.21
Asc. Acid (0.2%)	9.05	8.48	8.76	7.43	7.15	7.29	8.77	5.92	7.34
Mean	8.39	7.97		7.68	7.62		8.08	6.56	
	SEm±	CD (5%)		SEm±	CD (5%)		SEm±	CD (5%)	
Ascorbic acid (A)	0.145	0.445		0.082	0.251		0.466	NS	
Oil emulsion(B)	0.118	0.364		0.067	NS		0.381	1.173	
A X B	0.204	NS		0.115	NS		0.659	NS	
TSS (%)									
	Initial value = 28								
Without Asc. acid	29.00	30.50	29.75	30.50	32.50	31.50	32.00	35.00	33.50
Asc. Acid (0.1%)	29.00	30.00	29.50	30.00	33.00	31.50	32.00	35.00	33.50
Asc. Acid (0.2%)	29.00	30.00	29.50	30.00	32.00	31.00	31.00	34.00	32.50
Mean	29.00	30.16		30.16	32.50		31.66	34.66	
	SEm±	CD (5%)		SEm±	CD (5%)		SEm±	CD (5%)	
Ascorbic acid (A)	0.479	NS		0.612	NS		0.479	NS	
Oil emulsion(B)	0.391	NS		0.500	1.54		0.391	1.204	
A X B	0.677	NS		0.866	NS		0.677	NS	

the ripening process. It is concluded that (1. There is great potential in antioxidants to increase the post harvest shelf life. (2. Oil emulsion retards the shelf life and should not be used. (3. Ascorbic acid should be further used with different concentrations. (4. Some other antioxidants should also be tried.

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Incidence of ber butterfly, *Tarucus indica* in Bikaner district of Rajasthan.

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Ber is the most important arid fruit crop contributing over 80 per cent of the total fruit area in Bikaner district of western part of Rajasthan and cultivated in approximately 600 ha area in the district. During recent past, a decline in its productivity has been observed and there are many factors responsible for it. Among those, incidences of pests and diseases have been found significant. As many as 80 insect species have been found feeding on ber tree in India (Butani, 1979). Ber fruit fly is one of the major insect causing considerable damage to the crop. Beside it, ber butterfly, *Tarucus indica* was reported in Gujarat on ber (Shah et al, 1990) has been pest of minor significance. However, for last few years, it has become the most destructive pest of ber in the district. Keeping in view the severity the present investigation has been made in order to study a delicate relationship of weather factor to the larva population and incidence and current status of *T. indica* at different locations of Bikaner district. The observations of larvae were recorded at weekly interval at ber orchard Krishi Vigyan Kendra, Bikaner. Five full grown trees were selected at random. From each selected tree, 20 leaves were taken at random from top, middle and bottom parts. For counting larval population, the mean values of larvae infestation per tree for each orchard under given location were calculated. Meteorological data regarding temperature °C (maximum and minimum) per cent relative humidity (morning and evening) were obtained from the meteorological observatory, Agriculture Research Station, Beechwal, Bikaner from July to December. The weekly means of the above parameters were computed for the standard weeks. Correlation coefficients (r-value) for the larva of ber butterfly and abiotic factors responsible for seasonal variations were worked out. A comprehensive visits in all the ber growing area of district Bikaner were made during the last week of July, 08 and first week of Aug' 08 to study the incidence of *T. indica* at different locations. As many as 16 locations of Bikaner, Kolayat, Nokha, Dungargarh and Lunkaransar were visited and from each orchard, observations of larva per tree were recorded.

Seasonal incidence of ber butterfly was studied during 2008 under unsprayed condition. The population of larvae was recorded at weekly interval starting from first week of July to the last week of November. Data presented in Table-1 depicted that the larvae of ber butterfly appeared on the crop in the first week of July and built up in the population started from 31st standard week i.e. first week of August and continued to increase up to the last week of September and declined gradually till November. The population of larvae ranged from 1.10 to 10.20. The peak activity of larvae (10.20/tree) was recorded in first fortnight of August. The data on correlation between larval incidence and abiotic factor presented in Table-2 revealed that the maximum and minimum temperature showed positive correlation with larvae ($r=0.16$ and $r=0.61$). The morning relative humidity ($r=0.77$), evening relative humidity ($r=0.69$) and mean relative humidity ($r=0.76$) had significant positive correlation with larvae. The rainfall also showed positive correlation.

Data on incidence of *T. indica* in different locations of Bikaner district are presented in table 3. The average number of larva per tree was maximum (15.08) at Kolayat tehsil followed by Nokha (13.67), Dungargarh (11.93), Bikaner (10.58) and Lunkaransar (10.55). In Kolayat tehsil the average number of larvae per tree ranged from 11.12 to 18.46 where as these was recorded maximum in Diyatra village and minimum in maandal village. The range of larva per tree was observed 11.55 to 16.45 in Nokha tehsil followed by 9.20 to 15.73, 9.60 to 11.82 and 8.33 to 12.66 in Dungargarh, Bikaner and Lunkaransar tehsils, respectively. The minimum number of larvae was observed in Jagdevwala village of Lunkaransar tehsil.

Severity of the pest, a number of visits of different ber orchards of Bikaner and Kolayat tehsils were undertaken in the month of August, September and October to know the current status of the pest. Five orchards from three locations were visited. In each orchard, five full grown trees were selected at random. From each selected tree, 20 leaves were taken at random from top, middle and bottom parts. For counting caterpillar population, the mean

Table 1. Seasonal incidence of ber butterfly on ber

St. Week	Observation period	Temperature (°C)		Relative humidity (%)			Rainfall (mm)	Popu. of larvae/plant
		Max.	Min.	Mor.	Eve.	Mean		
27	03 July to 09 July	40.3	29.9	58.2	34.2	46.2	0.0	2.10
28	10 July to 16 July	37.7	27.4	76.2	42.4	59.3	4.9	2.20
29	17 July to 23 July	38.2	28.6	60.71	35.6	48.155	0.0	3.20
30	24 July to 30 July	39.8	29.3	63.3	33.14	48.22	7.0	3.00
31	31 July to 06 Aug.	35.7	27.1	85.9	70.1	78	98.5	8.10
32	07 Aug. to 13 Aug.	35.3	26.6	80.2	33.8	57	23.0	10.20
33	14 Aug. to 20 Aug.	36.6	26.7	83.7	49.7	66.7	0.0	8.22
34	21 Aug. to 27 Aug.	37.1	26.5	68.1	34.4	51.25	0.0	9.32
35	28 Aug. to 03 Sept.	37.2	27.1	72.8	35.6	54.2	0.0	7.60
36	04 Sept. to 10 Sept.	36.6	25.5	73.4	40.5	56.95	0.0	5.12
37	11 Sept. to 17 Sept.	36.8	24.9	74.1	35.8	54.95	0.0	4.14
38	18 Sept. to 25 Sept.	36.1	24.0	78.5	44.3	61.4	0.0	4.30
39	26 Sept. to 02 Oct.	36.1	24.2	74.6	33.2	53.9	0.0	2.70
40	03 Oct. to 09 Oct.	39.4	24.6	63.14	21.6	42.37	0.0	1.10
41	10 Oct. to 16 Oct.	38.6	23.4	56.6	18.8	37.7	0.0	0.00
42	17 Oct. to 23 Oct.	36.3	19.8	61.7	21.6	41.65	0.0	0.00
43	24 Oct. to 30 Oct.	36.1	17.8	46.5	13.8	30.15	0.0	0.00
44	31 Oct. to 06 Nov.	35.0	17.5	47.8	19.7	33.75	0.0	0.00
45	07 Nov. to 13 Nov.	34.4	16.5	50.71	15.4	33.055	0.0	0.00
46	14 Nov. to 20 Nov.	38.4	13.3	63.6	33.6	48.6	0.0	0.00
47	21 Nov. to 27 Nov.	28.0	8.9	53.29	18.2	35.745	0.0	0.00
48	28 Nov. to 04 Dec.	29.1	8.3	58.14	19.6	38.87	0.0	0.00

Table 2. Correlation co-efficients (r-value) between population of larvae and abiotic factors

S.No.	Weather parameters	Larval population/tree
1	Maximum temperature °C	0.16 NS
2	Minimum temperature °C	0.61*
3	Relative humidity morning %	0.77*
4	Relative humidity evening %	0.69*
5	Average relative humidity %	0.76*
6	Rainfall (mm)	0.31 NS

* Significant at 5 % level

NS = Non significant

Table 3. Level of incidence of ber butterfly *T. indica* in Bikaner district

S.No.	Tehsil	Location surveyed	Av. No. of larvae/tree	Overall mean & range/tehsil
1.	Bikaner	Pemasar	11.82 ± 2.11	10.58
		K.V. K, Farm	10.33 ± 1.92	(9.60 – 11.82)
		Hussinsar	9.60 ± 1.48	
2.	Kolayat	Diyatra	18.46 ± 1.11	15.08
		Kolayat	15.67 ± 1.67	(11.12 – 18.46)
		Mandal	11.12 ± 2.88	
3.	Nokha	Nokha	16.45 ± 2.15	13.67
		Deshnok	13.01 ± 1.45	(11.55 – 16.45)
		Maniyana	11.55 ± 1.18	
4.	Dungargarh	Dungargarh	15.73 ± 1.77	11.93
		Likhmidshar	10.88 ± 1.11	(9.20 – 15.73)
		Uppani	9.20 ± 2.25	
5.	Lunkaransar	Jagdevwala	8.33 ± 1.48	10.55
		Hansera	10.17 ± 1.32	(8.33 – 12.66)
		Khajuwala	12.66 ± 3.01	

values of caterpillar infestation per tree for each orchard under given location were calculated.

During the visit, it was observed that the butterflies appeared on ber in the month of May. June and remained active up to October and November. These butterflies can be seen on shrubs throughout the year. The butterflies (20 to 30mm in wing expanse) had dark brown black body and light blue coloured wings with dark spots. There were also dark black oval spots on head, thorax and wing base. The full grown caterpillars were generally heavy, flashy and slug like and yellowish green in colour. The head of the caterpillars was yellowish orange and red. The caterpillar constitutes the destructive stage. They feed on young leaves by scrapping chlorophyll in lines leaving behind a white transparent layer, later on resulted fall off leaves. In severely infested trees, the bearing capacity of trees was adversely affected. The

young plants were completely defoliated. Thus this pest could be devastating in the nursery and young plantation. The data on incidence of ber caterpillar are present in Table I revealed that average number of caterpillar per tree was maximum (18.46) at Diyatra in Kolayat tehsil followed by Beechwal (9.2) and Pemasar (6.6) of Bikaner tehsil. Keeping in view the severity of incidence of this pest there is need to work out effective control of this pest in ber orchard

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