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Pigeonpea-Lac insect interaction: Effect of lac culture on grain yield and biochemical parameters in pigeonpea

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Abstract

Present experiment was conducted to know the impact of pigeonpea-lac insect interaction for the production of raw lac/scrapedlac as well as grain yield and protein quality in seeds. On the basis of broodlac and scrapedlac yield, five genotypes of pigeonpea *viz*., IPA 8-2, Bahar, Assam local, Acc.no. 591139 and RCMP 5 were identified promising for lac production. Rearing of lac insect on pigeonpea reduced 100 seed weight (13.03%) and grain yield per plant (12.08%) significantly but no significant reduction was observed on crude protein content in seeds (1.02%). Biochemical traits measured in mature leaves in inoculated and control plants revealed that reducing sugar (RS), chlorophyll 'a' and total chlorophyll decreased after lac insect inoculation but non-reducing sugar, total sugar (TS), proline and malondialdehyde increased after insect inoculation. The biotic stress rendered due to lac insect (free phenol) and abiotic stresses due to high temperature (malondialdehyde) did not significantly affect scrapedlac yield. High chlorophyll components along with high metabolites (RS and TS) supported high scrapedlac yield. Hence, farmers can cultivate lac on this host plant to get good raw lac and grain yield without losing the crude protein quality of grain. The loss in grain yield is compensated by additional income from raw lac. The profit obtained from raw lac along with grain will be much higher than sole crop for grain.

Key words: Pigeonpea, lac insect, interaction, grain yield, biochemical traits

Introduction

Lac insect is one of the most important beneficial insect in the world. Protective cover of the insect, the resinous secretion of economic importance, is widely used in different areas in diverse fields [1, 2]. Lac insect is distributed in Tropical Asia such as India, Pakistan, Bangladesh, Myanmar, Thailand, South China, Taiwan, *etc*. Two species of lac insect i.e., *Kerria lacca* in India and *K. chinensis* in China and Thailand, are most exploited species for industrial purpose. Lac is one of the unique products of plant-insect interaction. It consists of resin, pigment, wax and traces of proteinaceous materials, inorganic salts and a few odoriferous substances [3]. Lac wax is used in coating fresh fruits of apple and orange, so that their shelf-life can be increased. Aleuritic acid, the principal material in the resin, is extensively used as a starter in perfumery industry [4]. Lacquer, a product derived from lac, is useful in realizing an elegant sheen to painted wooden products. In recent years, lac has beeb used in coating pharmaceutical products for absorption in the hind gut and not in the stomach of human. The water soluble lac dye has been used as a cosmetic in human decoration, for dyeing wool and silk and to stain leather [5]. The production of more resin has become a necessity due to demand of lac resin as raw material in the industries. The current prosperity in the lac market provides an incentive for farmers to increase lac production [6].

There are more than 400 recorded hosts for lac insect, but traditionally, most important hosts are *kusum* (*Schluchera oleosa* (Lou.) Oken.), *ber* (*Ziziphus mauritiana* Lam.) and *palas* (*Butea monosperma* Lam.) in Eastern India and pigeonpea (*Cajanus cajan*) in north-east India. New hosts and strains of lac insect are being investigated to enhance production. It will improve the livelihood of small and marginal farmers in lac growing areas. Pigeonpea, a perennial member of the family *Fabaceae*, is an important crop for small scale farmers. The pigeonpea was identified as lac host long back in China, Vietnam and India. In 1950s pigeonpea was identified as a favourate host for lac insect in Yunnan

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province of south west China [7]. On-farm lac production with pigeonpea has recently emerged and spread in the Northern part of The Lao Peoples' Democratic Republic (Lao PDR) as a result of increasing demand of lac from China [8]. These species has also been reported as promising host in North-East India [9].

Pigeonpea is grown in 191 thousand hectare land in Jharkhand with average productivity of 955 kg/ha during 2012-13 [10]. To harness the potential for lac production, Indian Institute of Natural Resins and Gums (formerly Indian Lac Research Institute, ILRI) had initiated trials way back in 1978. It was concluded that *Baisakhi* crop of *rangeeni* strain of *K. lacca* (Ker) was most suitable for lac as well as grain production in late maturing varieties. Germplasm from Manipur and ICRISAT along with local cultivar *Bahar* performed better for lac production [11]. Although 32% reduction in grain yield occurs due to lac cultivation on it, but profit obtained from lac was much higher than the crop loss. About 25% more income can be generated as compared to sole crop [12]. Stages of lac culture on pigeonpea *i.e.,* initial settlement density; sex differentiation and lac incrustation at maturity are presented in Fig. 1. Hence the impact of pigeonpea-lac insect interaction was assessed in medium and late maturity group of pigeonpea to know the potential of broodlac/scrapedlac as well as grain yield and protein quality in seeds.

Materials and methods

Twenty six germplasm/ land races/varieties of pigeonpea were procured from AICRP on pigeon pea (12), ICAR-RC-NEH Manipur Centre (4), ICRISAT, Hyderabad (5), NBPGR, Ranchi (1), BAU (2) and local collection (2). Birsa Arhar 1 and Bahar were well adapted varieties and recommended for cultivation for grain and used as check for medium and late maturity group, respectively. The experiment was conducted at Institute Research Farm (IRF) of ICAR-IINRG, Ranchi, Jharkhand during June 2010 to July 2012.

Fig. 1. Stages of lac culture on pigeonpea, a. settlement of crawlers, b. sex differentiation stage, c. lac encrustation at maturity

Field was prepared with deep summer ploughing followed by two ploughing with cultivator before onset of monsoon. Compost/farm yard manure @10 tons/ hectare was applied in the field after summer ploughing and ridges were made. To minimize the effect of acidic soil on plant growth lime was applied in lines @ 3 quintal/ hectare at sowing time. Chemical fertilizers were used as per recommendation (N: P: K @ 25: 50: 25 kg/ha) for North Eastern Plain Zone (NEPZ) in ridge at sowing time. After receiving first rain fall all genotypes were sown in ridges. Each plot was of 5m length with row to row and plant to plant spacing of 75 cm with three replications. Paired row per genotype was spaced at 150 cm for better aeration to lac insects. On an average 11850 plant populations per hectare were maintained. To minimize initial weed competition, Pendimethalin 30 EC was sprayed @ 3.2 liters/hectare in 500-600 liters of water just after sowing in presence of moisture in the soil. Weeds were managed by inter culturing (hoeing and weeding) during initial growth period. The plants were pinched (removing growing tips of the seedlings) during early stage of growth to enhance the production of primary branches [7]. For lac production, five-months-old plants were used. Plant growth was adequate during the month of October-November for lac insect inoculation. For a summer crop of *Rangeeni* strain of lac insect (*Kerria lacca*) was inoculated @ 25 gram broodlac per plant in one row and another row was kept as control (without lac inoculation) to know the effect of lac inoculation on grain yield parameters. For this purpose 5–9 suitable branches with more than 0.8 cm diameter were selected. Since the broodlac is tied below

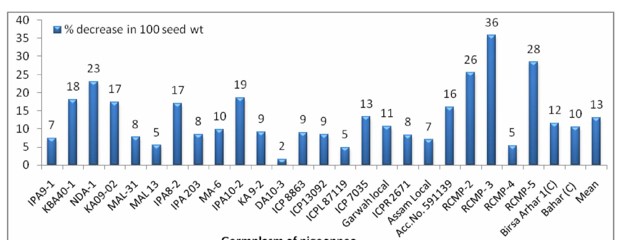

Fig. 2. Loss in 100 seed weight after lac inoculation

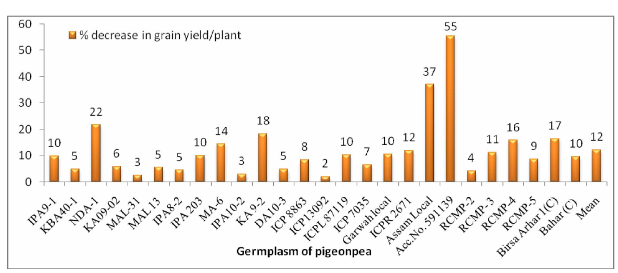

Fig. 3. Loss in grain yield per plant after lac inoculation

the first primary branch, larvae of the lac insect swarm spontaneously to settle down on the branches, insert proboscis in phloem sap and become static. They feed on plant sap and secrete resin which is deposited around the branches or stem. Pigeon pea was irrigated frequently at 10-15 days interval after harvest of grain in April- May to maintain photo-transpiration equilibrium.

Data were recorded for grain quality traits like 100 seed weight, grain yield per plant and crude protein (percent) in seeds in control and inoculated conditions separately. Broodlac was ready for harvesting at maturity during June-July. Harvested broodlac was weighed for each variety. The resinous cover was scraped off from the twigs, weighed as scrapedlac. For biochemical analysis, leaf samples were collected around 11:00 a.m. and plunged in boiling 95 percent ethanol. Subsequently, leaf samples were analyzed for reducing, non-reducing, total sugars. There were three replications for each determination. The sugars were determined by Nelson's arsenomolybdate [13] method using improved copper reagent of Somogyi [14]. Non-reducing sugar was calculated by subtracting reducing sugar from total sugar. Soluble protein was estimated by Lowrey's [15] method. Estimation of total (free) phenols was done by Folin-Ciocalteau[16] method. Chlorophyll 'a', chlorophyll 'b', total chlorophyll and carotenoids were estimated by Hiscox and Israelstam [17] DMSO method and their levels were calculated according to the formula of Lichtenthaler [18]. Malondialdehyde was determined as a product of lipid peroxidation in the leaves as per Minotti and Aust [19]. Data were calculated for one and two factors analysis to know the genetic variability, interaction of lac insect on genotype of pigeonpea and correlation coefficient between scrapedlac and plant biochemical parameters.

Results and discussion

Assam local, Acc.no. 591139 and RCMP 5 produced significantly higher broodlac than check Bahar. On the other hand, IPA 8-2, Acc. No. 591139 and Bahar were having significantly higher scrapedlac yield than check Birsa Arhar 1. Altogether, five genotypes of pigeon pea *i.e.*, IPA 8-2, Bahar, Assam local, Acc.no. 591139 and RCMP 5 were identified promising for broodlac/ scrapedlac production (Table 1). Earlier report revealed that germplasm from Manipur and ICRISAT along with local cultivar Bahar had better lac production potential [11]. Rearing of lac insect on pigeonpea reduced 100 seed weight (13.0%) and grain yield per plant (12.1%) significantly. Loss in 100 seed weight ranged from 2 to 36% and that of grain yield from 2 to 55%. DA 10-3 had minimum reduction in 100 seed weight followed by ICPL 87119, RCMP 4, MAL 13 (Fig. 2). The loss in grain yield per plant was the lowest in ICP 13092 (2%) after lac inoculation followed by MAL 31 (3%), IPA 10-2 (3%) and RCMP 2 (4%) and the highest in Acc. No. 591139 (55%) followed by Assam local (37%) (Fig. 3). Average loss in grain yield was 12.1% among 26 germplasm. Sharma and Ramani12 also observed 32% reduction in grain yield due to lac cultivation on it, but profit obtained from lac was much higher than the crop loss. About 25% more income could be generated as compared to sole crop for grain even if lac produced was sold as raw lac. About 50-60 thousand rupees additional income per hectare can be generated inspite of reduction in pulse yield.

Protein plays an important role in biochemical system, working as signal molecule in various pathways. When protein content in seeds of host plant was analyzed in inoculated *vis-a-vis* un-inoculated condition, decrease in seed protein (1.02%) was observed in inoculated condition; but the loss in seed protein was non-significant. The non significant difference is due to the fact that upon inoculation lac insect activates protein synthesis in the leaves to produce more protein for defense as well as for development of both, the lac insect and the plant. Almost negligible reduction in crude protein was recorded in *Bahar*, ICP 13092, ICPL 87119, MAL 13, ICP 7035 and KBA 40-1 (Fig. 3). Some changes in this trait were observed in IPA 203 (3.9%), ICP 8863 (2.8%) and NDA 1 (2.5%).

Broodlac production capability varies widely due to inter and intra host differences which is attributed mainly due to nutrition supplying capacity of the host [20]. The phloem is a well-known target of sucking and piercing insects that utilize the transported sap as their major nutrient source [21]. It was observed in present study that the reducing sugar in the leaf was found decreased in pigeonpea genotypes upon inoculation with lac insect. The total sugar and the non-reducing

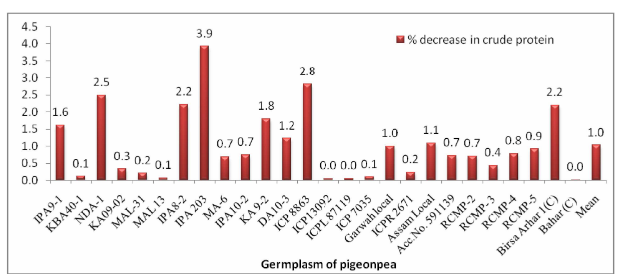

Fig. 4. Loss in crude protein % in seeds after lac inoculation

Table 1. Broodlac and scrapedlac yield along with other attributing traits in pigeon pea varieties

Varieties	Broodlac yield/plant (g)	Scrapedlac/ plant (g)	100-seed wt (g)		Grain wt /plant (g)		Crude protein % in seeds	
			Con[1]	Ino[2]	Con	Ino	Con	Ino
IPA9-1	100.7	11.7	10.2	9.5	31.0	28.2	9.6	8.0
KBA40-1	81.7	14.1	12.4	10.5	51.6	49.2	9.6	9.5
NDA-1	60.3	18.3	14.4	11.7	19.6	16.1	10.7	8.2
KA09-02	37.5	13.6	14.2	12.1	63.7	60.2	10.0	9.6
MAL-31	78.2	11.9	12.6	11.7	51.0	49.7	10.1	9.9
MAL 13	83.3	13.8	11.6	11.0	32.8	31.1	10.8	10.8
IPA8-2	116.7	23.7+	12.4	10.6	32.2	30.8	13.5	11.3
IPA 203	45.2	12.3	14.2	13.1	26.1	23.7	16.5	12.6
MA-6	31.7	14.7	12.3	11.2	11.9	10.4	12.4	11.7
IPA10-2	55.9	14.1	13.4	11.3	23.9	23.2	12.1	11.3
KA 9-2	116.7	16.4	13.1	12.0	26.4	22.3	15.0	13.2
DA10-3	75.4	18.1	12.5	12.3	62.6	59.7	13.6	12.3
ICP 8863	58.3	14.0	12.1	11.1	24.3	22.4	11.3	8.5
ICP13092	46.4	14.9	14.0	12.9	39.7	38.9	9.5	9.5
ICPL 87119	59.2	12.3	12.8	12.2	30.2	27.4	10.0	10.0
ICP 7035	35.3	8.9	13.6	12.0	27.6	25.9	9.6	9.5
Garwah local	31.5	14.0	14.5	13.1	42.1	38.1	11.3	10.3
ICPR 2671	100.3	14.4	11.8	10.9	34.6	30.9	10.4	10.2
Assam Local	164.8*	13.9	10.7	10.0	22.2	16.2	10.8	9.7
Acc.No. 591139	121.9*	22.7+	15.9	13.7	40.4	26.0	11.4	10.7
RCMP-2	100.0	12.8	11.8	9.4	24.5	23.5	10.6	9.9
RCMP- 3	63.6	12.4	12.1	8.9	17.8	16.0	9.7	9.3
RCMP-4	100.0	10.5	9.9	9.4	23.4	20.2	11.4	10.6
RCMP-5	159.5*	13.1	12.2	9.5	18.9	17.4	10.9	10.0
Birsa Arhar 1(C)	28.1	17.8	10.6	9.5	24.7	21.2	10.2	8.0
Bahar (C)	117.5	22.0+	11.6	10.5	27.3	24.9	9.8	9.8
Mean	**79.6**	**14.9**	**12.5**	**11.1**	**30.5**	**27.6**	**11.2**	**10.2**
Range	28.1-164.8	8.9-23.7	9.9-15.9	8.9-13.7	11.9-63.7	10.4-60.2	9.5-16.5	8.0-13.2
CD Variety	11.18	0.84	0.27		0.53		1.06	
CD Inoculation			0.08		0.15		N/A	
CD Interaction			0.39		0.75		1.5	

1. con=no lac inoculation (control), 2. ino= lac inoculation**,** ** and * = significant at 1 and 5 % probability level, respectively
*= significantly higher than Bahar, + = significantly higher than Birsa Arhar 1

sugar increased in case of lac insect inoculation in majority of the pigeonpea genotypes. The increase/ decrease in the sugar content of the pigeonpea (Fig. 5 a, b, c) suggests that the photosynthetic activity was significantly influenced by infestation of lac insect. The sugar (carbohydrates) synthesis is induced by the lac insect infestation, thereby increasing the source of carbon in the form of sugar in the sap. This facilitates lac insect survival as well as host plants [21]. This finding seems to be an important way by which sap-sucking insects increase their sugar concentration in sap of the host plant for its own survival. The free phenol content

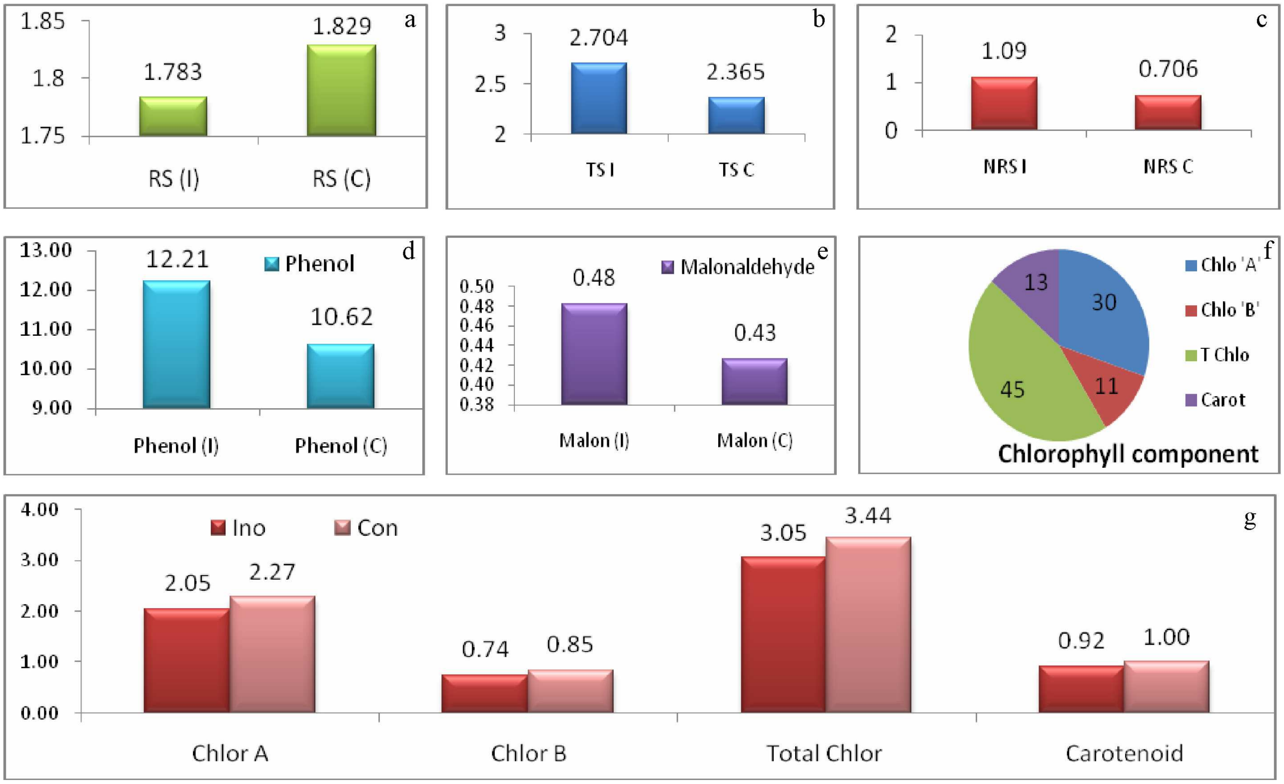

Fig. 4. (a-g): Biochemical parameters in germplasm of pigeonpea in inoculated and control conditions; a) Redusion sugar; b) told sugar; c) Non-reducing sugar; d) free phenol; e) malonaldehyde; f) composition of chlorophyll and g) chlorophyll components. RS= reducing sugar, NRS= nonreducing sugar, TS= total sugar, Ino= inoculated, Con= control

in leaves in germplasm of pigeonpea increases upon lac insect inoculation (Fig. 5 d). The transcripts belonging to secondary metabolic processes such as phenyl propanoid biosynthesis, flavanoids, and aromatic compounds were up-regulated (expressed) during later phase of infestation [22].

Malondialdehyde is an indicator of oxidative stress. Malondialdehyde content in leaves of genotypes of pigeonpea increased upon lac insect inoculation (Fig. 5 e). Changes in atmospheric temperature are often varying suddenly and plant cannot adjust to these sudden changes and are damaged beyond recovery [23]. High temperature results in the desiccation of a plant and disturbs the balance between photosynthesis and respiration. Though malondialdehyde is a bye product of lipid peroxide which is caused due to high temperature, the increase in malondialdehyde due to lac infestation needs further study. Chlorosis is the most obvious plant injury symptom on leaves after lac insect feeding and is indicative of chlorophyll loss. Such effect may be clarified by the significant reduction in chlorophyll 'a' and 'b' contents as well as carotenoids determined in the leaves of inoculated plants of pigeonpea (Figure 5 g). The decrease in the photosynthetic pigments may be due to the inhibition of pigment biosynthesis which may results from the alteration in mineral nutrition or lack of assimilates which drain towards the insect or to the effect of reactive oxygen species on these pigments [24]. Chlorophyll 'a' had maximum share in expression of component followed by Chlorophyll 'b' and Carotenoids (Fig. 5 f).

Scrapedlac is raw material for industries. Initial settlement density of crawlers, initial mortality and female to male sex ratio are some of the important lac attributing traits but lac yield also depends on biochemical property of plant from where lac insects feed. Scrapedlac yield had positive and significant association with reducing sugar, total sugar, chlorophyll A and total chlorophyll. Total sugar was significantly correlated with reducing sugar, non-reducing sugar, chlorophyll A, chlorophyll B, total chlorophyll and scrapedlac (Table 2). Two stress indicators free phenol and malondialdehyde although associated with each other but they had no significant association with other

Table 2. Correlation coefficient among raw lac and biochemical traits in pigeonpea

Traits	RS	NRS	TS	Phenol	Malon	Chlor A	Chlor B	T Chlor	Carot	SY
RS	1.000									
NRS	0.204^{NS}	1.000								
TS	0.735^{**}	0.814^{**}	1.000							
Phenol	0.306^{NS}	0.162^{NS}	0.294^{NS}	1.000						
Malon	0.195^{NS}	-0.449^{NS}	-0.195^{NS}	0.596^{*}	1.000					
Chlor A	0.641^{*}	0.392^{NS}	0.652^{*}	-0.148^{NS}	-0.208^{NS}	1.000				
Chlor B	0.376^{NS}	0.499^{NS}	0.569^{*}	-0.228^{NS}	-0.378^{NS}	0.859^{**}	1.000			
T Chlor	0.593^{*}	0.350^{NS}	0.594^{*}	-0.209^{NS}	-0.293^{NS}	0.965^{**}	0.926^{**}	1.000		
Carot	0.698^{**}	-0.059^{NS}	0.373^{NS}	-0.163^{NS}	-0.048^{NS}	0.842^{**}	0.640^{*}	0.835^{**}	1.000	
SY	0.712^{**}	0.305^{NS}	0.634^{*}	0.378^{NS}	-0.108^{NS}	0.570^{*}	0.500^{NS}	0.624^{*}	0.552^{NS}	1.000

RS=reducing sugar, NRS= non-reducing sugar, TS= total sugar, Malon=malondialdehyde, Chlor A= chlorophyll 'A', Chlor B= chlorophyll 'B', T Chlor= total chlorophyll, Carot=Carotenoid, SY= scrapedlac yield. ** and *= significant at 1% and 5% probability levels, respectively.

traits. Here, high metabolites and chlorophyll component supported scrapedlac yield in pigeonpea.

It can be concluded that pigeonpea-lac insect interaction plays significant role in lac production. Selected germplasm of pigeonpea *i.e*., IPA 8, Bahar, Assam local, Acc.no. 591139 and RCMP 5 can be used as potential host for lac production as well as pulse in sub tropical region of India. High chlorophyll components along with high metabolites supported high scrapedlac yield. Farmers can rear lac insect on this host plant to get raw lac and grain without losing the crude protein quality of grain. The loss in grain yield is compensated by additional income from raw lac. Hence, more income can be generated by lac cultivation on pigeonpea as compared to sole crop for grain.

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