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Isolation and identification of effective *Rhizobium* germplasm for increasing productivity of Kulthi (*Dolichos biflorus* L.) under climatic conditions of Chhattisgarh

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Abstract
A field experiments were conducted at Sarguja, Bastar and Raipur C.G. continuous three year to select location specific *Rhizobium* isolates(s) for Horse gram local name Kulthi (Halba) through systematic screening of local *Rhizobium* germplasm comparing with local checks. In addition to newly *Rhizobium* germplasm of 313 new *Rhizobium* isolates(s) were isolated from soils of Sarguja, Korba and Bastar districts under the present investigation. On the basis of Biological Nitrogen Fixing performance 10 best isolates were selected through screening of the 313 newly collected germplasm under glass house conditions. Above 10 best local isolates and local check as native Rhizobia with General Recommended Dose of fertilizers were considered for studies in field conditions. Study under glass house sand culture devoid of nitrogen source conditions clearly revealed the variation in the symbiotic effectiveness of the tested rhizobial isolates. The nitrogen fixing efficiency of national and local isolates were studied in terms of parameters like plant height, nodulation, biomass accumulation, plant nitrogen content and its uptake by Horse gram var. AK-21. The keeping in view, seed yield of Horse gram was recorded 486.5, 467.04, 462.59, 453.14, 444.80, 442.02, 436.46, 425.34 425.34 and 414.22 kg/ha due to inoculation with local isolates numbered, 11, 56, 41, 2, 51, 12, 67, 47, 59, and 10 respectively. The seed yield 139.00 kg/ha was recorded the lowest and 486.5 kg/ha was observed the highest among all isolates under the field study in absolute control and isolate no 11, respectively.

Keywords: Isolation, identification, effective *rhizobium*, increasing productivity, climatic, Chhattisgarh

Introduction
Horse gram (locally known as 'kulthi' in Chhattisgarh,'Gahat' in Sikkim) is the unexploited legumes of the tropics and subtropics grown mostly under dry-land agriculture and important pulse crop taken in tribal belt of the Chhattisgarh. In Chhattisgarh total cultivated area of this crop is about75 thousand hectares, out of this, nearly 37 thousand hectares are situated only in Bastar Divison representing an important kulthi belt in the state. This crop is very popular among the marginal and poor farmers which includes tribals and backwards population of Chhattisgarh. The important kulthi growing districts of Chhattisgarh are Jagdalpur, Kanker and Sarguja with the production of about 13.80 thousand tonnes with a productivity of less than 5. 0 quintal ha^{-1} (Anonymous, 2003) [1] the productivity per unit area of this crop is very low mainly because of sub optimal nutrition (Gupta *et al;* 2000 a) [2, 9, 10].

Horse gram is sharp, bitter and hot. It has tremendous nutritive and medicinal values, which is not yet tapped and exploited properly. Some of its medicinal properties *viz* it is known as beneficial in cough breathing problem due to phlegms, flatulation, hicups, stones and fever. It also eliminates germs and worms. It causes impurities of bile and blood. It also causes inflammation and checks sweating. Drinking semi liquid solution of horse gram powder cures flatulation. If horse beans mixed with powdered dry ginger, asafetida and "veed salt" is taken it cures the pain of the stomach, if it is used in food it cures disease of the stomach. If the water in which horse gram had been soaked for the whole night (and is mashed in the same water in the morning) is taken twice daily, then it cures "stones". The use of horse gram in the diet relieves the pain of dry piles. Drinking the semi liquid mixture of powdered horse gram and powdered black pepper cures sore throat. Its use in the diet is good for women as their menstruation is purified. On the other hand it is harmful for pregnant woman or a person suffering from plethora or tuberculosis and also causes the formation of excessive bile.

One of the cheapest and easiest methods to add organic matter to the soil is companion cropping of horse gram along with rice crop. This system of simultaneous growing of horse gram along with rice is suited to areas where dry sowing is practiced and subsequent submergence of the field is anticipated with the onset of south-west monsoon. After the sowing of rice seeds, seeds of horse gram are sown at 6 to 7 kg/ha. No separate fertilizers are required for horse gram which is raised for green manure purpose. With the onset of south-west monsoon, the rice field gets submerged and self-incorporated into the soil. In normal rainfall years; at the time of submergence of the field, the horse gram crop may be at the age of about six weeks and at the active vegetative phase. Trials conducted have revealed that the system of companion cropping of horse gram along with rice crop is more suited to areas receiving delayed rainfall. It is estimated that in normal monsoon seasons this method add about 2 to 3 MT of green manure to the soil. If the rainfall is delayed, the quantity of green manure added to the soil is increased. The system is more acceptable to farmers than growing cowpea along with rice as the horse gram crop may not smother the main rice crop even if the south-west monsoon is delayed than normally expected. The weed problem is also reduced substantially. The aim of the proposed study was to obtain more yield of kulthi crop in Chhattisgarh in same field by same amount of financial input through the use of location specific effective *Rhizobium* under INM system of sustainable agriculture.

Methods and Materials

The field experiments were conducted with Horsegram (var. A.K.21) at farmer's field of Sarguja, Bastar and Raipur. The field experiment was laid out as per the layout plan in an *entisols*. The number of treatments were 12, replicated thrice in randomized block design (plot size, 4 x 3 mts^2 and row to row spacing was 30 cm). The same treatments were applied in all the three experimental sites on farmers' field at different locations on Horsegram during *kharif* 2007 under the present investigations. The field was prepared by repeated ploughing followed by leveling. Nitrogen (N), phosphorus (P2O5) and Potassium (K2O) were applied in all the treatments except absolute control as basal at the rate of 20:40:20 kg/ha through urea, single super phosphate and muriate of potash, respectively. Certified healthy Horsegram seeds were treated before sowing with thiram @ 3 gm/kg of seed. After fungicidal treatment, seeds were inoculated with rhizobial inoculum. The method of seed inoculation and the quantity of applied rhizobial inoculum per seed by was same as mentioned earlier.

Results

Plant height study

Data presented in table 1 revealed that plant height of Horse gram at 45 DAS increased significantly over control due to inoculation of most of the isolates under study. At 45 DAS, among different treatments, plant height recorded as the highest due to inoculation of isolate no.-47 followed by isolate no.-41 in Sarguja, isolate no.-56 in Bastar and isolate no.-12 in Raipur. However, the difference among these isolates expressed in terms of plant height was statistically at par .The rest of the isolates also performed significantly better over control. Out of tested 10 local isolates, all isolates showed significantly higher plant height over local control, except isolate no. 2 in case of Raipur district. While, application of fertilizers gave at par values in various treatments of plant height as compared with control values at different locations. The lowest value of plant height was 15.77 cm, while the highest was 48.43 cm due to inoculation in control and isolate - no.47, respectively. These findings were clearly supported by Garg, (2010) [7], Singh *et al.*, (2011) [22], they mentioned that growth of legume plants can be increased significantly byinoculation with effective local rhizobial isolates.

Dry matter yield study

Results on dry matter yield indicated in table-1 revealed that at 45 DAS, The dry weight of plants increased significantly due to inoculation of all the isolates over control. Amongst them, the highest dry weight was recorded due to inoculation of isolate no.-56 followed by isolate no-59. The lowest value of dry weight per plant was 12.02, while highest was 46.85 g per plant due to inoculation of control and isolate - no.41, respectively. At 45DAS, all the 60 isolates tested under the study gave higher nodulation plant growth possibly because of low and less effective native population of horse gram *Rhizobium* in the soil. Similar findings were also reported in by Barik and Mukherjee (1995), Katre *et al.* (1997) [12] and Prasad (1997) [15].

Number of Nodules

The data shown in table-2 revealed that number of effective nodules, counted at 45 DAS increased significantly in the plants raised from seed inoculated with all isolates under field study. At 45 DAS, all the local isolates, under investigations produced significantly higher number of nodules per plant over control. Application of fertilizers also gave significant response with respect to effective number of nodules over control. The number of nodules /plant increased significantly which ranged from 20.67 to 48.00. The lowest no. of nodule / plant was 11.67 while the highest was 48.33 due to inoculation of control and isolate - no.47, respectively. Similar trends were also observed under field conditions of Raipur farmers'. Number of nodules per plant increased significantly from 25.33, to 49. 67 in different treatments treated with varying isolates. Similar findings were reported by Gupta *et al.* (2000) [2, 9, 10] and Gupta *et al.*, (2005) [11], Salod Kumar (2004), Garg (2010) [7] and Sheikh *et al.*, (2012), Saleh *et al.*, (2013).They mentioned that number of (nodules/plant) can be increased by inoculation with effective rhizobial strains. This observation is also supported by findings of Many other researchers also gave opinion similar to that findings of present investigation

Dry weight of nodules

The data presented in table-2 revealed that dry weight of nodules per plant at 45 DAS increased significantly as compared with control in the plants raised from seed inoculated with all isolates under field study. At 45 DAS, all the local isolates produced higher dry weight of nodules per plant significantly over control. However, these isolates were at par among themselves with respect to oven dry weight of nodules. Application of fertilizers gave significant response over control. The highest dry weight of nodules per plant was recorded 16.18, due to inoculation of local isolates numbered 56, 41, 47, 12, 11, 59, 2, 51 67 and 10 respectively under Sarguja farmers' field. The lowest value of dry weight of nodules was 4.18 while the highest was 16.18 due to inoculation of control and isolate – no. 56, respectively. At Jagdalpur farmers the dry weight of nodules per plant recorded significantly higher i.e.16.18, 15.04 and 14.75 mg. over control due to inoculation of local isolate number 41, 47

and 56, respectively. The lowest value of dry weight of nodules per plant was 5.24 while, the highest was 16.18 due to inoculation of control and isolate - no.56, respectively. Similar trends were observed in field condition of Raipur. The lowest value of nodule per plant was 5.11, while the highest was 21.74 mg due to inoculation of control and isolate - no.12, respectively. Similar findings were reported by Gupta *et al.* (2000) [2, 9, 10] and Gupta *et al.*, (2005) [11], Salod Kumar (2004), Garg (2010) [7] and Sheikh *et al.*, (2012), Saleh *et al.*, (2013).They mentioned that number of (nodules/plant) can be increased by inoculation with effective rhizobial strains.

Nitrogen uptake study

Nitrogen uptake is associated with yield parameters of crop. The data on N-uptake are presented in table 3. It is clearly indicated from table that nitrogen uptake by seeds of Horsegram increased significantly due to inoculation with different local isolates. The nitrogen uptake in seeds was found significantly higher due to inoculation of isolate no. 56 (21.02%) followed by isolate no. 11 (20.87%) and isolate no. 41 (19.61%) as compared with rest of isolates; however, they were at par among themselves. The lowest N uptake in seed was recorded 4.84 kg per ha in control and the highest uptake value of 21.02 kg per ha as recorded in isolate no. 56. At Jagdalpur farmers' field Nitrogen uptake by seeds in Horsegram increased significantly from 14.53 to 19.63 kg per ha in different isolates. The lowest value of nitrogen uptake in seeds was 5.94, while the highest was 19.63 kg per ha due to inoculation of were control and isolate no.56, respectively and Raipur farmers' field. The significantly higher nitrogen uptake of 20.83 and 18.54 kg per ha was recorded due to isolate no. 56 and 11, respectively which were at par to each other. The lowest value of nitrogen uptake in seeds was 5.37 while the highest was 20.83 kg per ha due to inoculation of control and isolate - no.56, respectively.

The data nitrogen uptakes in straw of Horsegram are presented in table 3. The data indicated that nitrogen uptake of straw followed the similar trend as in case of seeds. The nitrogen uptake of straw in Horsegram increased significantly from 3.72 to 6.31 kg per ha due to inoculation of local isolates, under Sarguja farmer's field. The lowest value of nitrogen uptake in straw was 1.14 while, the highest was 6.31 kg per ha due to inoculation of control and isolate no.41, respectively. Nitrogen uptake by straw in horse gram increased significantly from 3.89 to 5.26 kg per ha due to inoculation of local isolates under Jagdalpur farmers' field. The lowest value of nitrogen uptake in straw was 1.59 kg/ha while the highest was 5.26 kg per ha due to inoculation of control and isolate no. 56, respectively. The nitrogen uptake of straw in Raipur was recorded 5.27, 5.20, 4.22, 4.13, 4.10, 3.82, 3.66, 3.59, 3.42 and 3.12 kg per ha due to inoculation of local isolates numbered from 41, 51, 12, 11, 56, 59, 47, 2, 67 and 10, respectively. The lowest value of uptake in straw was 1.33 kg per/ha while the highest was 5.27 kg per ha due to inoculation of control and isolate no.41, respectively.

The data on total nitrogen-uptake by horse gram are presented in table 3 and depicted in Fig. 10 reveled that the nitrogen uptake was recorded significantly higher in isolate no.56, at Jagdalpur and Raipur locations., However at Sarguja, isolate no. 41 recorded higher nitrogen uptake, both the isolates were at par to each other. The control recorded the lowest nitrogen uptake as compared with local isolates. Results of present investigation are also in confirmation with the findings of Prasad (1997) [15] who got better local isolates with those of recommended national and international isolates through systematic screening of local *Rhizobium* germplasm. Findings of the present investigation are also close to observations of and Gupta *et al.*, (2000) [2, 9, 10] a; and Garg (2010) [7]. They clearly mentioned that N-uptake by legume can be increased by use of specific *Rhizobium* isolates.

Yield Study

The data on seed yield of Horsegram at harvest are presented in table-5 revealed that yield of Horsegram increased significantly due to inoculation with all isolates over control under study. At Sarguja farmer's field, the highest seed yield was recorded due to inoculation with isolate numbered 11 followed by isolate no. 56 among all tested isolates. The seed yield increased significantly from 414.22 to 486.50 kg/ha due to different treatments. The seed yield 139.00 kg/ha was recorded the lowest and 486.5 kg/ha was observed the highest among all isolates under the field study in absolute control and isolate no 11, respectively. The similar trends were observed under Jagdalpur farmer's field. The seed yield in Horsegram increased significantly from 380.30 to 436.17 kg/ha due to inoculation with different promising isolates. The seed yield of Horsegram was recorded 436.17, 434.35 420.34, 410.33, 400.32, 395.32, 392.81, 390.31, 380.30 and 380.30 kg/ha due to inoculation with local isolates numbered from 56, 11, 2, 41, 12, 51, 59, .67, 47 and 10 respectively at Jagdalpur. The lowest value of seed yield was 172.64 kg/ha while, the highest was 436.17 kg/ha due to inoculation of local control and isolate no .56. seed yield observed under farmer's field condition of Raipur. The seed of yield Horsegram increased significantly from 385.73 to 462.87 kg /ha due to varying isolates. The seed yield of Horsegram was recorded 462.87, the lowest seed yield was recorded 154.29 kg/ha while, the highest seed 462.87 kg/ha due to inoculation of local control and isolate no.56, respectively.

The data on straw yield of Horsegram presented in Table 4 showed that straw yields at Sarguja farmer's field was recorded highest due to inoculation with isolate number 11 amongst all better 10 isolates, which was followed by isolate no 56. The straw yield increased significantly from 588.19 to 690.83 kg/ha due to different local isolates. The lowest straw yield was recorded 197.38 kg/ha and the highest observed 690.83 kg/ha, in absolute control and isolate number 11, respectively. Straw yield observed in farmer's field condition of Jagdalpur. The straw yield of Horsegram increased significantly from 509.61 to 584.47 kg/ha due to inoculation of better isolates as compared with control. The lowest straw 231.33 kg/ha while the highest was 584.47 kg/ha due to inoculation of local control and isolate no 56 respectively, Like similar trends were observed in field condition of Raipur farmer's. Field the straw yield in Horsegram increased significantly from 520.73 to 624.87 kg/ha in different treatments. The lowest straw yield in control was 208.29 kg/ha while, the highest was 624.87 kg/ha isolate no. 56 due to inoculation. Similar results were also reported by Sharma *et al.*, (2001) [20], Ashraf *et al.*, (2003) [3], showed that seed inoculation with *rhizobium* strain significantly increased mungbean grain yield. Sharma *et al.*, (2001) [20], Kumaga and Ofori, (2004) [13], Malik *et al.*, (2006) [14], Our findings are in conformity with that of Aslam *et al.*, (2006), who reported that application of *Rhizobium* inoculum generally increased yield components. Fatima *et al.*, (2007) [5] and Fatima *et al.*, (2008) [6], they reported that legume seeds inoculation and phosphorus application significantly increased grain yield. Similar results were also obtained by Sahid *et al.*, (2009) [19].

Table 1: Influence of different local rhizobial isolates on Plant height and dry weight of plant (Horsegram) grown under different locations

Isolates No.	Plant height at 45 DAS (cm)			Dry weight of plant at 45 DAS (g/plant)		
	Sarguja	Jagdalpur	Raipur	Sarguja	Jagdalpur	Raipur
2	43.90	31.67	24.40	33.56	31.16	39.33
10	30.04	36.67	35.00	31.07	30.26	42.50
11	42.11	40.00	39.70	31.74	30.87	28.30
12	43.08	39.00	46.03	31.22	32.45	44.57
41	45.11	42.67	44.00	33.61	33.33	45.26
47	45.77	45.67	48.43	43.65	44.03	46.01
51	34.53	35.33	45.93	30.39	40.96	32.92
56	44.83	44.67	43.42	62.69	60.92	46.85
59	38.82	42.67	34.62	53.19	52.89	46.76
67	34.51	40.33	35.17	36.76	34.03	26.85
Fertilizers	28.73	23.16	22.97	24.93	23.70	21.07
Absolute control	20.45	21.67	15.77	16.96	14.11	12.02
CD 5%	8.65	8.28	8.89	3.97	6.58	7.90

Table 2: Influence of different local rhizobial isolates on nodulation and dry weight of nodules of Horsegram grown under field Conditions.

Isolates No.	No. of effective nodules/ plant at 45 DAS at			Dry wt. of nodules/plant (mg)		
	Sarguja	Jagdalpur	Raipur	Sarguja	Jagdalpur	Raipur
2	43.33	38.00	32.00	11.93	11.59	9.15
10	40.67	40.00	33.67	11.14	10.75	10.52
11	42.00	38.00	37.67	13.85	14.27	14.27
12	43.00	33.33	25.33	14.36	14.36	21.74
41	40.33	36.33	38.33	15.04	15.04	15.04
47	48.00	48.33	43.00	14.84	14.75	14.75
51	43.67	44.00	48.67	11.91	11.69	14.34
56	47.33	45.33	49.67	16.18	16.18	15.89
59	43.00	40.00	38.00	13.29	13.29	8.00
67	20.67	34.67	30.33	11.27	11.50	7.17
Fertilizers	13.67	28.00	21.33	7.38	7.15	5.92
Absolute control	07.00	11.67	15.00	4.18	5.22	5.11
CD 5%	3.44	6.49	9.09	5.22	9.15	4.63

Table 3: Influence of different local rhizobial isolates on N uptake in seed and straw of Horse gram grown under different locations.

Isolates No.	N uptake in seeds (kg/ha)			N uptake in straw (kg/ha)			Total N uptake of Horsegram (kg/ha)		
	Sarguja	Jagdalpur	Raipur	Sarguja	Jagdalpur	Raipur	Sarguja	Jagdalpur	Raipur
2	18.49	16.90	16.11	4.83	4.53	3.59	23.31	21.43	19.70
10	16.11	14.79	15.00	4.00	3.96	3.12	20.11	18.76	18.13
11	20.87	18.46	18.54	4.84	4.95	4.13	25.71	23.41	22.67
12	18.74	17.34	17.87	4.71	4.65	4.22	23.45	21.98	22.09
41	19.61	17.40	17.23	6.31	4.66	5.27	25.92	22.06	22.49
47	16.76	14.98	15.70	4.11	4.02	3.66	20.87	19.00	19.36
51	17.08	15.30	16.98	5.50	4.10	5.20	22.58	19.40	22.18
56	21.02	19.63	20.83	4.31	5.26	4.06	25.33	24.89	24.89
59	17.99	16.99	17.08	4.23	4.55	3.82	22.22	21.54	20.89
67	15.84	14.53	15.31	3.72	3.89	3.42	19.56	18.42	18.72
Fertilizers	15.04	13.05	15.11	3.53	3.50	3.00	18.57	16.55	18.11
Absolute control	4.84	5.94	5.37	1.14	1.59	1.33	5.98	7.53	6.70
CD 5%	2.27	4.51	2.27	0.60	1.21	0.54	2.86	5.72	2.52

Table 4: Influence of different local rhizobial isolates on N uptake in seed and straw yield and protein content in Horse gram grown under different locations.

Isolates No.	Seeds yield (kg/ha)			Straw yields (kg/ha)		
	Sarguja	Jagdalpur	Raipur	Sarguja	Jagdalpur	Raipur
2	453.14	420.34	408.87	643.46	563.25	551.97
10	414.22	380.30	385.73	588.19	509.61	520.73
11	486.50	434.35	437.16	690.83	582.03	590.16
12	442.02	400.32	416.58	627.67	536.43	562.39
41	462.59	410.33	406.30	656.88	549.84	548.50
47	425.34	380.30	398.58	603.98	509.61	538.09
51	444.80	395.32	442.30	631.62	529.72	597.10
56	467.04	436.17	462.87	663.20	584.47	624.87
59	425.34	392.81	403.73	603.98	526.37	545.03
67	436.46	390.31	421.73	619.77	523.02	569.33
Fertilizers	414.22	365.29	370.30	588.19	489.49	499.90
Absolute control	139.00	172.64	154.29	197.38	231.33	208.29

CD 5%	34.60	44.60	36.48	81.96	68.10	30.40

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