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Assessment of nodulation potential in mini-core genotypes and land races of chickpea

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ABSTRACT

Symbiotic nitrogen fixation (SNF) is a sustainable alternative for nitrogen supply for plants in agriculture. Past efforts to enhance SNF in chickpea through inoculation with improved rhizobia were partially successful. Hence, there is an urgent need to identify nodulation variants among the mini-core and races accessions of chickpea. In the present study, a total of 211 mini-core lines, 68 land races and 3 checks were evaluated for nodulation variants under greenhouse conditions at ICRISAT, Patancheru, India and IIPR, Kanpur, India. The seeds of all accessions were inoculated with IC-76, a nodulating bacteria, on both locations. When the potting material was pasteurized, the organic carbon had reduced from 0.49% to 0.29% whereas no significant difference was noted in total N and available P contents. At 45 days after sowing, the mini-core lines of chickpea were categorized into 6 types, based on nodulation capability (rating 0-5, where 0=no nodules; while 5 = maximum nodules) at both ICRISAT and IIPR locations. A direct correlation was obtained between nodule numbers and shoot and root weights. Further, some lines were found common at both the locations for particular category of nodulation rating.For instance, the lines ICC-2580, ICC-2990, ICC-3421 and RSG-888 were found fitting in rating 5 while lines ICC-6294 and ICC-9002 in rating 1. A similar trend of nodulation variants were also found in the land races of chickpea. A total of 35 chickpea mini-core lines and six land races were found common for nodulation variants at both locations.

Key words: Chickpea, Land races, Mini-core genotypes, Nitrogen fixation, Nodulation

About 70% of all nitrogen used by agriculture is supplied by inorganic fertilizers with the reminder supplied by symbiotic nitrogen fixation (SNF) (Galloway *et al*. 2008). Nitrogen supplied through SNF is less likely to volatilize or leach than that supplied as inorganic fertilizer (Jensen and Hauggaard-Nielsen 2003). The most important nitrogen fixing agents in agricultural systems are the symbiotic associations between legumes and rhizobia. The grain legumes (often called as pulses such as chickpea, pigeonpea, beans, lentil and groundnut) are often targets for increased SNF because of their high food value and suitability in crop rotations (Biabani *et al*. 2011). Further, grain legumes meet a large part of their own nitrogen demand through SNF and also partially contribute to the nitrogen requirement of the following crop. Past efforts to enhance SNF through inoculation with rhizobia were only partially successful as nodulation and nitrogen fixation is a complex process and is dependent on compatibility of both rhizobia and legume symbiosis under various environmental and soil conditions. Although, some selected efficient strains of rhizobia and legumes have shown encouraging results under field conditions, there is a need for consistent and positive influence on nodulation and nitrogen fixation under variable conditions.

Chickpea (*Cicer arietinum* L.) is the third most important grain legumes crop in the world, after bean and soybean, with a total production of 14.2 million tonnes from an area of 14.8 million ha and a productivity of 960kg ha^{-1} (FAOSTAT 2013), providing high-quality proteins for human and animal nutrition. Rhizobia specific to chickpea were reported to fix 40% 60 kg N ha^{-1}year^{-1} (Herridge *et al*. 2008). Many factors determine the rate of nitrogen fixation of which host plants pay a vital role. Breeders have been reticent to incorporate SNF as a breeding objective due to lack of good selection tools, and without clear evidence of the potential of success among other multiple breeding objectives. Hence, the strategies to improve SNF should include selection of effective rhizobial strains, rhizosphere soil available nitrogen management and crop breeding.

Gene banks such as that of ICRISAT (Patancheru, India) Svalbard Global Seed Vault (Norway) and the US National Plant Germplasm System (Beltsville, USA) maintain and evaluate highly diverse germplasm collections in order to preserve plant biodiversity and supply genetic resources for crop improvement to institutions around the world. Plant genetic resources are widely used in breeding programming for identifying sources of resistance or susceptible to insect pests and diseases (Sudini *et al*. 2015). Chickpea germplasm contains several thousands of lines and characterization of these lines for a particular trait takes several years to complete. Hence, Upadhyaya and Ortiz (2001) proposed a "mini core collection" concept in which selection of 1% of the entire collection of the germplasm using all the available information such as origin, geographical distribution, characterization and evaluation of morphological, agronomical and quality traits. Mini-core collections with diverse agronomic traits are used extensively in plant breeding programmes (Upadhyaya *et al*. 2010). Screening of such chickpea mini-core lines can be an important aspect for identifying promising donors to SNF. Nodulation variants in chickpea among the mini-core and land races accessions will help the breeders in the selection as well as breeding processes as a line with SNF trait has additional advantage. The main aims and objectives of this investigation were to determine the nodulation potential in a diverse subset (mini-core) of available chickpea germplasms at ICRISAT and IIPR and due to determine whether variations in nodulation occur within this mini-core subset.

MATERIALS AND METHODS

The chickpea mini-core collection consisting of 211 accessions (Upadhyaya and Ortiz 2001) was developed based on geographic distribution and qualitative traits from 1956 accessions of the core collection of chickpea, representing 16,991 accessions available in the ICRISAT gene bank (Upadhyaya *et al*. 2001). The qualitative traits considered in selecting mini-core included 28 early maturing accessions, 39 high grain yield accessions, 16 large seed sized Kaubli type accessions, 12 salinity tolerance accessions, 18 drought tolerance accessions, 5 high temperature tolerance accessions, 67 wilt resistant accessions, 6 dry root rot resistant accessions, 3 *Ascochyta* blight resistant accessions, 55 *Botrytis* tolerant accessions, 18 multiple disease resistant accessions and 5*Helicoverpa* tolerant accessions (Kashiwagi *et al*. 2005,Pande *et al*. 2006, Vedez *et al*. 2007 and Upadhyaya *et al*. 2007a, 2007b) and 68 land races. Three chickpea cultivars (BG-256, Subhra and RSG 888) were also used as standard checks. The details of these accessions including accession number, origin and seed weight are given in Table 1.

Table 1. The table of chickpea mini-core (1%211) and land races (212%279) collections used in the study

S. No.	Acc. No. ICC-	Origin	Seed weight (mg)	S. No.	Acc. No. ICC-	Origin	Seed weight (mg)	S. No.	Acc. No. ICC-	Origin	Seed weight (mg)
1	67	India	162	38	2277	Iran	266	75	5613	India	188
2	95	India	125	39	2507	Iran	159	76	5639	India	152
3	283	India	171	40	2580	Iran	266	77	5845	India	126
4	440	India	149	41	2629	Iran	139	78	5878	India	127
5	456	India	140	42	2720	Iran	92	79	5879	India	198
6	506	India	186	43	2884	Iran	138	80	6263	Russia & CISs	306
7	637	India	159	44	2919	Iran	198	81	6279	India	158
8	708	India	142	45	2969	Iran	100	82	6293	Italy	133
9	762	India	126	46	2990	Iran	185	83	6306	Russia & CISs	240
10	791	India	115	47	3218	Iran	100	84	6537	Iran	126
11	867	India	161	48	3230	Iran	116	85	6571	Iran	74
12	1052	Pakistan	113	49	3325	Cyprus	145	86	6579	Iran	142
13	1083	Iran	182	50	3362	Iran	108	87	6802	Iran	159
14	1098	Iran	108	51	3421	Israel	145	88	6811	Iran	135
15	1161	Pakistan	115	52	3512	Iran	184	89	6816	Iran	162
16	1164	Nigeria	163	53	3631	Iran	102	90	6874	Iran	159
17	1180	India	158	54	3761	Iran	101	91	6877	Iran	240
18	1194	India	222	55	3776	Iran	150	92	7184	Turkey	103
19	1205	India	170	56	3946	Iran	104	93	7255	India	332
20	1230	India	241	57	4182	Iran	120	94	7272	Algeria	365
21	1356	India	160	58	4418	Iran	137	95	7308	Peru	196
22	1392	India	330	59	4463	Iran	126	96	7315	Iran	297
23	1397	India	205	60	4495	Turkey	145	97	7323	Russia & CISs	178
24	1398	India	211	61	4533	India	261	98	7441	India	148
25	1422	India	201	62	4567	India	223	99	7554	Iran	
26	1431	India	199	63	4593	India	169	100	7571	Israel	288
27	1510	India	187	64	4639	India	183	101	7668	Russia & CISs	240
28	1710	India	125	65	4657	India	112	102	7819	Iran	246
29	1715	India	158	66	4814	India	131	103	7867	Iran	202
30	1882	India	159	67	4841	Morocco	225	104	8058	Iran	180
31	1915	India	253	68	4872	India	238	105	8151	USA	156
32	1923	India	101	69	4918	India	217	106	8195	Pakistan	132
33	2065	India	131	70	5135	India	144	107	8261	Turkey	330
34	2072	India	135	71	5337	India	267	108	8318	India	245
35	2210	Algeria	122	72	5383	India	192	109	8350	India	201
36	2242	India	128	73	5434	India	165	110	8384	India	170
37	2263	Iran	154	74	5504	Mexico	287	111	8522	Italy	146

(Table 1 contd…)

S.No.	Acc.No. ICC	Origin	Seed weight (mg)	S.No.	Acc. No. ICC	Origin	Seed weight (mg)	S.No.	Acc.No. ICC	Origin	Seed weight (mg)
112	8607	Ethiopia	154	149	12328	Cyprus	440	186	14831	India	179
113	8621	Ethiopia	169	150	12492	ICRISAT	152	187	15264	Iran	310
114	8740	Afghanistan	265	151	12537	Ethiopia	124	188	15294	Iran	280
115	8855	Afghanistan	204	152	12654	Ethiopia	159	189	15333	Iran	386
116	8950	India	152	153	12726	Ethiopia	140	190	15406	Morocco	384
117	9002	Iran	119	154	12824	Ethiopia	129	191	15435	Morocco	290
118	9137	Iran	318	155	12851	Ethiopia	161	192	15510	Morocco	292
119	9402	Iran	126	156	12866	Ethiopia	122	193	15518	Morocco	278
120	9586	India	144	157	12916	India	157	194	15567	India	169
121	9643	Afghanistan	154	158	12928	India	175	195	15606	India	139
122	9755	Afghanistan	198	159	12947	India	251	196	15610	India	190
123	9848	Afghanistan	164	160	13077	India	200	197	15612	Tanzania	218
124	9862	Afghanistan	164	161	13124	India	394	198	15618	India	165
125	9895	Afghanistan	189	162	13187	Iran	220	199	15697	Syria	260
126	9942	India	157	163	13219	Iran	174	200	15802	Syria	268
127	10341	Turkey	300	164	13283	Iran	435	201	15868	India	174
128	10393	India	141	165	13357	Iran	224	202	15888	India	158
129	10399	India	195	166	13441	Iran	145	203	16207	Myanmar	196
130	10755	Turkey	170	167	13461	Iran	206	204	16261	Malawi	151
131	10885	Ethiopia	380	168	13523	Iran	270	205	16269	Malawi	134
132	10945	India	142	169	13524	Iran	138	206	16374	Malawi	165
133	11121	India	136	170	13599	Iran	190	207	16487	Pakistan	115
134	11198	India	144	171	13628	Unknown	161	208	16524	Pakistan	174
135	11284	Russia & CISs	169	172	13764	Iran	180	209	16796	Portugal	300
136	11378	India	148	173	13816	Russia & CISs	240	210	16903	India	193
137	11498	India	180	174	13863	Ethiopia	139	211	16915	India	195
138	11584	India	127	175	13892	Ethiopia	116	212	2679	Iran	
139	11627	India	112	176	14051	Ethiopia	130	213	2737	Iran	
140	11664	India	156	177	14077	Ethiopia	139	214	3239	Iran	
141	11764	Chile	283	178	14098	Ethiopia	158	215	3391	Iran	
142	11879	Turkey	210	179	14199	Mexico	313	216	3410	Iran	208
143	11944	Nepal	149	180	14402	ICRISAT	184	217	3582	Iran	
144	12028	Mexico	248	181	14595	India	216	218	3892	Iran	
145	12037	Mexico	180	182	14669	India	187	219	4093	Iran	76
146	12155	Bangladesh	152	183	14778	India	132	220	4363	Iran	
147	12299	Nepal	90	184	14799	India	161	221	4853	NOI	
148	12307	Myanmar	176	185	14815	India	187	222	4958	India	252

The nodulation potential of mini-core genotypes and land races of chickpea was conducted in a completely randomized design (CRD) under glass house conditions with the potting material of sand and black soil (1:1). The potting material was pasteurized twice at the interval of 48 h at 70 °C for one h. The potting material was analysed for total N, available P and OC before and after sterilization. The seeds of all the chickpea accessions were surface sterilized with sodium hypochlorite (2.5% for 5 min) and rinsed thoroughly with sterilized water. The sterilized seeds were transferred to into yeast extract mannitol (YEM) broth cultured with nodulating bacteria specific for chickpea, IC 76, and incubated for 50 min. At the end of incubation, the seeds were sown in the pots (3 seeds/pot and thinned to one after a week). The plants were irrigated once in every 3 days with 30 ml of sterilized deionised water. At 45 days after sowing, nodule number and dry weight of shoot and root were determined. Nodulation capacity is presented on rating scale of 0%5 (IIPR: 0 = No nodule; 1= 0.1 to 6 nodule; 2= 7 to 12 nodules; 3= 13 to 18 nodules; 4= 19 to 24 nodules; 5= >24 nodules; ICRISAT: 0 = No nodule; 1= 0.1 to 1 nodule; 2= 1 to 2 nodules; 3= 2 to 4 nodules; 4= 4 to 6 nodules; 5= >6 nodules).

The data were subjected to ANOVA (GenStat 10.1 version 2007, Lawes Agricultural Trust, Rothamsted Experimental Station) and the significance was tested at $p < 0.01$ and 0.05.

RESULTS AND DISCUSSION

The economic and environmental importance of legume crops is largely due to their ability to fix atmospheric dinitrogen in a symbiosis with specific bacteria through the process of nodulation and N_2 fixation.The present study has evaluated the nodulation variation of chickpea mini-core collection and also land races at ICRISAT and IIPR. A detailed indication on accession number, origin and single seed weight for all the chickpea accession is given in Table

(Table 1 contd…)

NOI= No information

S. No.	Acc. No.	Origin	Seed weight (mg)
223	4991	India	151
224	5221	India	105
225	6294	Iran	140
226	6875	Iran	
227	7052	Iran	62
228	7305	Afghanistan	
229	7326	NOI	
230	7413	India	165
231	8200	Iran	
232	8515	Greece	100
233	8521	Italy	
234	8718	Afghanistan	215
235	8752	Afghanistan	140
236	9418	Iran	
237	9434	Iran	
238	9590	Egypt	122
239	9636	Afghanistan	
240	9702	Afghanistan	156
241	9712	Afghanistan	
242	9872	Afghanistan	
243	10018	India	
244	10466	India	
245	10693	Turkey	
246	10685	Turkey	
247	10939	India	100
248	11279	Pakistan	
249	11303	Chile	
250	12324	NOI	
251	12379	Iran	
252	13719	Iran	
253	15248	NOI	
254	15614	NOI	
255	15762	NOI	
256	15785	NOI	
257	16654	NOI	
258	IG 5909	NOI	
259	IG 6044	NOI	
260	IG-6047	NOI	
261	IG-6055	NOI	290
262	IG-6067	NOI	
263	IG-6154	NOI	
264	IG-6343	NOI	
265	IG-6905	NOI	370
266	IG-7087	NOI	395
267	IG-7296	NOI	280
268	IG-10309	NOI	225
269	IG-10419	NOI	260
270	IG-10500	NOI	
271	IG-10569	NOI	
272	IG-10701	NOI	151
273	IG-69438	NOI	
274	IG-72070	NOI	
275	IG-72109	NOI	200
276	IG-73458	NOI	
277	ICC-5912	NOI	
278	ICCV-07110	NOI	
279	ICCV-92944	NOI	285

1. It was observed that, most of the accessions are from India followed by Iran and Afghanistan, the Asian countries but it also includes genotypes from other continents. Table 2 & 3 shows the nodulation data for the mini-core chickpea collection at IIPR and ICRISAT, respectively. In the study conducted at IIPR, only one chickpea accession ICC 16207 was observed to be non-nodulating, but the same was observed to be nodulating in ICRISAT with the rating of 1. Similarly, 39 chickpea accessions were observed to be non-nodulating in ICRISAT; but they were able to produce nodules at IIPR in which 16 chickpea accessions were observed to produce nodules at the rating of 3 followed by 12 and 5 chickpea accessions producing nodules at the rating of 4 and 5, respectively. Differences in the nodulation capacity of other chickpea accessions were also observed on ICRISAT and IIPR. In the context of land races also similar trend was noticed (Table 4). Land races IG 10701, IG 6905, IG 7296 produced nodules at the rating of 4 at IIPR; but found to be non-nodulating in ICRISAT. This difference might be due to the variation in the soil types and its level of compaction (Girvan *et al.* 2003, Siczek and Lipiec 2011). Although genetic factors of rhizobium and leguminous crops are the base of the successful nodulation yet the environmental factors such as soil water content, aeration and temperature also have the impact on nodulation and further N_2 fixation.

In IIPR, 85 chickpea accessions were found to produce nodules at the rating scale of 3, followed by 68 accessions at scale of 4 and 65 accessions at the scale of 5. In the context of ICRISAT, 62 accessions produced nodules at the scale of 1 followed by 53, 40, 39 and 27 accessions produced nodules at the scale of 3, 2, 4 and 5 respectively. It is interesting to note that, some of the lines were found common in both the locations for particular category of nodulation rating. For instance the lines ICC 2580, ICC 2990, ICC 3421 and RSG 888 were found fitting in rating 5, while line ICC 6294 and ICC 9002 in rating 1 for both the locations. A total of 35 chickpea mini-core lines and 7 land-races were found common in both ICRISAT and IIPR (Table 5&6). In addition, check entries showed higher nodulation rating at both ICRISAT and IIPR.

In both IIPR and ICRISAT locations, a direct correlation was obtained between nodule numbers and shoots and root growth of chickpea (Table 2& 3). A similar trend was noticed on land-races also (Table 4). In contrast, report of Biabani *et al.* (2011) on global chickpea mini-core collection states that, there was a correlation between shoot and total plant weight with plant nitrogen fixed but not with nodule numbers.

Mineral contents (OC%, total N and available P) of soils used as potting material were analyzed before and after sterilization and presented in Table 7. The OC was

Table 2. Nodulation variants of mini-core lines of chickpea at IIPR, Kanpur, India

Nodule Ranke*	Number of lines	Nodules (plant^{-1})	Root weight (g plant^{-1})	Shoot weight (g plant^{-1})	Accession numbers
0	1	0	0.055	0.211	ICC-16207
1	6	8	0.070	0.205	ICC-1180, -4495, -6294, -9002, -10755, -16654
2	21	11	0.090	0.193	ICC-867, -1710, -2065, -2242, -2720, -3761, -3776, -3892, -3946, -4182, -4657, -4814, -4918, -5221,-5879, -7315, -8515, -9590, -11121, -11498, -11764
3	85	15	0.097	0.217	ICC-95, -440, -456, -506, -708, -752, -762, -791, -1052, -1098, -1161, -1164, -1205,- 1422, -1915, -1923, -2072, -2210, -2263, -2277, -2629, -2679, -2884, -3218, -3362, -3631, - 4093, -4363, -4418, -4841, -4872, -5135, -5383, -6293, -6537, -6571, -6579, -6811, -6875, -7326, -7571, -7668, -7819, -8085, -8195, -8261, -8318, -8350, -8384, -8607, -8621, -8855, -9402, -9636, -9643, -9702, -9712, -10399, -11198, -11279, -11378, -11584
4	68	21	0.111	0.255	ICC-283, -637, -1194, -1392, -1882, -2507, -2919, -2969, -3230, -3325, -3410, -4463, -4533, -4593, -4639, -5337, -5504, -5613, -5639, -5845, -5878, -6279, -6306, -6816, -6874, -6877, -7184, -7255, -7272, -7308, -7413, -7441, -7867, -8151, -8521, -8718, -8740, -8950, -9137, -9586, -9942, -10018, -10341, -10393, -10466, -10693, -10885, -11284, -11627, -12379, -12654, -12726, -12824, -12866, -12947, -13219, -13523, -13524, -14051, -14077, -14815, -15294, -15406, -15567, -15868, -16487, BG-256, **Subhra** (checks)
5	65	29	0.117	0.280	ICC-67, -1083, -1356, -1397, -1398, -1431, -1510, -2580, -2737, -2990, -3239, -3421, -4567, -4958, -4991, -5434, -6263, -6802, -7052, -7305, -7323, -7554, -9418, -9434, -9755, -9862, -9895, -10685, -10945, -11944, -12037, -12307, -12328, -12537, -12916, -13077, -13124, -13187, -13283, -13357, -13441, -13461, -13599, -13816, -13892, -14199, -14402, -14595, -14669, -14799, -14831, -15264, -15333, -15606, -15610, -15618, -15697, -15762, -15802, -15888, -16269, -16374, -16903, -16915, **RSG-888** (check)

* Rank used for samples collected from IIPR: 0 = No nodule; 1= 0.1 to 6 nodule; 2= 7 to 12 nodules; 3= 13 to 18 nodules; 4= 19 to 24 nodules; 5= >24 nodules

Table 3. Nodulation variants of mini-core lines of chickpea at ICRISAT Patancheru, India

Nodule Rank*	Number of lines	Nodules (plant-1)	Root weight (g plant-1)	Shoot weight (g plant-1)	Accession numbers
0	39	0	0.347	0.337	ICC-440, -456, -708, -867, -1098, -1180, -1205, -1230, -1392, -1397, -1431, -1710, -4958, -5383, -5845, -6571, -6579, -6874, -7255, -7308, -7413, -7571, -7819, -8058, -8261, -8607, -8621, -8740, -9862, -9942, -11198, -11378, -11627, -12851, -14051, -15406, -15510, -15567, -15802
1	62	1	0.353	0.349	ICC-67, -95, -762, -791, -867, -1052, -1083, -1422, -2307, -2629, -4093, -4991, -5135, -5221, -5613, -5639, -5878, -6294, -6811, -6816, -6877, -7052, -7315, -7441,-7668, -8151, -8318, -8384, -8752, -9002, -9702, -9755, -9848, -9895, -10341, -10393, -10939, -11121, -11498, -11664, -11944, -12155, -12299, -12328, -12537, -12726, -12854, -12866, -12916, -12928, -13283, -13523, -13863, -14077, -14098, -14595, -14831, -15606, -15697, -15868, -15888, -16207
2	40	2	0.402	0.402	ICC-283, -506, -1164, -1356, -1510, -2065,-6410, -6263, -6802, -7184, -7323, -8195, -8350, -8718, -8855, -8950, -9137, -9402, -9434, -9586, -9590, -10399, -11284, -11584, -11764, -11879, -12947, -13441, -13461, -13628, -13816, -14199, -14402, -14778, -14799, -15264, -15333, -15618, -16654, -16796
3	53	4	0.425	0.405	ICC-637, -1194, -1398, -1715, -1882, -2072, -3239, -3582, -4363, -4593, -4918, -5434, -5879, -6293, -6537, -7305, -7554, -8515, -8521, -8522, -9418, -9643, -9712, -10693, -10755, -10885, -10945, -12037, -12324, -12379, -12492, -12654, -13077, -13219, -13357, -13524, -13599, -13764, -13892, -14815, -14669, -15248, -15435, -15610, -15612, -15762, -15785, -16261, -16374, -16524,-16903, -16915, **BG-256** (check)
4	39	7	0.439	0.460	ICC-1923, -2242, -2679, -2720, -2737, -2969, -3325, -3391, -3892, -4418, -4495, -4533, -4567, -4639, -4657, -4814, -4841, -4853, -5337, -6279, -6306, -6875, -7272, -7326, -8200, -9636, -9872, -10018, -10685, -11279, -11303, -12028, -13124, -13187, -13719, -15518, -15294, -16269, **Subhra** (check)
5	27	12	0.440	0.469	ICC-1161, -1915, -2210, -2263, -2277, -2507, -2580, -2884, -2919, -2990, -3218, -3230, -3362, -3421, -3512, -3631, -3761, -3776, -3946,-4182, -4463, -4872, -5504, -10466, -15614, -16487, **RSG-888(check)**

Table 4. Nodulation variants and shoot and root weight in land races lines of chickpeaat both IIPR and ICRISAT locations

Accession numbers	IIPR				ICRISAT			
	Nodules rating*	Nodules (plant-1)	Root Weight (g plant-1)	Shoot Weight (g plant-1)	Nodules rating*	Nodules (plant-1)	Root Weight (g plant-1)	Shoot Weight (g plant-1)
IG-5905	4	18.3	0.108	0.466	1	0.3	0.633	0.582
IG-5912	2	5.7	0.041	0.121	4	6.0	0.415	0.412
IG-6044	4	17.7	0.074	0.339	4	5.3	0.715	0.359
IG-6047	5	20.3	0.159	0.318	5	8.3	0.447	0.564
IG-6055	2	10.0	0.091	0.261	2	1.3	0.465	0.451
IG-6067	3	12.0	0.130	0.389	3	4.7	0.548	0.557
IG-6154	2	9.7	0.047	0.144	5	7.0	0.490	0.485
IG-6443	5	25.0	0.215	0.505	4	5.7	0.760	0.485
IG-6905	4	17.3	0.144	0.399	0	0.0	0.470	0.650
IG-7110	5	33.0	0.261	0.459	4	6.0	0.602	0.411
IG-7296	4	20.0	0.175	0.095	0	0.0	0.597	0.725
IG-10309	1	0.7	0.100	0.139	1	0.7	0.770	0.405
IG-10419	3	10.3	0.096	0.289	1	0.3	0.548	0.454
IG-10500	5	23.0	0.707	0.091	5	7.7	0.490	0.425
IG-10569	5	25.0	0.072	0.251	3	3.7	0.663	0.385
IG-10701	4	18.7	0.128	0.362	0	0.0	0.547	0.563
IG-69438	5	28.3	0.112	0.402	4	5.3	0.563	0.486
IG-72070	3	12.7	0.054	0.239	4	6.0	0.558	0.451
IG-72109	4	18.3	0.135	0.366	2	1.3	0.578	0.355
IG-73458	4	18.3	0.116	0.312	4	4.7	0.483	0.459
IG-92944	5	24.0	0.142	0.508	2	1.7	0.732	0.701

*Rating used for IIPR (per plant): 0 = No nodule; 1 = 0.1 to 5 nodules; 2 = 5 to 10 nodules; 3 = 10 to 15 nodules; 4 = 15 to 20 nodules; 5 = >20; Rating used for ICRISAT (per plant): 0 = No nodule; 1 = 0.1 to 1 nodule; 2 = 1 to 3 nodules; 3 = 3 to 5 nodules; 4 = 5 to 7 nodules; 5 = >7 nodules

Table 5. Nodulation variants of min-core lines found common at both IIPR and ICRISAT locations

Nodulation Rating*	Number of lines	Accession numbers
1	2	ICC-6294, ICC-9002
2	3	ICC-2065, ICC-9590, ICC-11764
3	15	ICC-2072, ICC-4363, ICC-6293, ICC-6537, ICC-9643, ICC-9712, ICC-12324, ICC-12492, ICC-13764, ICC-15248, ICC-15435, ICC-15612, ICC-15785, ICC-16261, ICC-16524
4	11	ICC-2969, ICC-3325, ICC-4533, ICC-4639, ICC-5337, ICC-6279, ICC-6306, ICC-7272, ICC-10018, ICC-13719, ICC-15294
5	4	ICC-2580, ICC-2990, ICC-3421, RSG-888

*Ratings used for IIPR: 0 = No nodule; 1= 0.1 to 6 nodules; 2 = 7 to 12 nodules; 3 = 13 to 18 nodules; 4 = 19 to 24 nodules; 5= >24 nodules; Ratings used for ICRISAT: 0 = No nodule; 1 = 0.1 to 1 nodule; 2 = 1 to 2 nodules; 3 = 2 to 4 nodules; 4 = 4 to 6 nodules; 5 = >6 nodules

Table 6. Nodulation variants of land races lines found common at both IIPR and ICRISAT locations

Nodulation Rating*	Number of lines	Accession numbers
1	1	IG-10309
2	1	IG-6055
3	1	IG-6067
4	2	IG-6044, IG-73458
5	2	IG-10500, IG-6-47

* Rating used for IIPR (per plant): 0 = No nodule; 1 = 0.1 to 5 nodules; 2 = 5 to 10 nodules; 3 = 10 to 15 nodules; 4 = 15 to 20 nodules; 5 => 20 nodules; Rating used for ICRISAT (per plant): 0 = No nodule; 1 = 0.1 to 1 nodule; 2 = 1 to 3 nodules; 3 = 3 to 5 nodules; 4 = 5 to 7 nodules; 5 = >7 nodules

Table 7. Mineral contents of soils used in the greenhouse for mini-core experiments

Treatment	Organic carbon (%)	Total N (ppm)	Available P (ppm)
Before autoclave	0.49	602	1.60
After autoclave	0.29	623	1.72
Mean	0.39	612	1.66
SEm(±)	0.003	0.7	0.003
LSD(%)	0.063*	12.7*	0.063*
CV%	1	1	1

*= Statistically significant at 0.05

reduced from 0.49 to 0.29%. Sterilization has no significant effect on total N and available P content. Micronutrients including Fe, Zn, Cu, Mn, Mg and total P estimated in chickpea stover (Table 8) for some of the selected chickpea accessions categorized like good nodulation,poor nodulation, good shoot weight, poor shoot weight, good shoot weight and good nodule number, poor shoot weight and poor nodule number.Mineral content was varied between the accessions irrespective of the nodulation status. Besides all these variations, further studies on plant fixed nitrogen are critical for recommending the selected line for breeding programs.

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Table 8. Micro-nutrient analysis of stover of mini-core lines of chickpeagrown under greenhouse conditions (45 DAS)

Accession Numbers	Selection basis	Fe Zn (ppm)	Cu (ppm)	Mn (ppm)	Mg (ppm)	Total (ppm)	P (%)
ICC-3218	GN	222	40	7.77	274	4796	0.20
ICC-3761	GN	230	42	6.82	311	3984	0.18
ICC-5504	GN	258	48	6.80	291	3525	0.17
ICC-15614	GN	199	42	6.95	299	3786	0.15
ICC-1180	PN	278	69	7.44	366	3084	0.12
ICC-9862	PN	238	67	7.67	347	4118	0.19
ICC-11284	PN	143	66	8.15	379	3755	0.17
ICC-13219	PN	202	57	7.38	668	4338	0.19
ICC-15697	PN	197	55	7.01	389	3914	0.17
ICC-1161	GSH	849	72	8.17	353	4013	0.12
ICC-2065	GSH	435	52	6.95	411	3960	0.12
ICC-4567	GSH	410	57	6.50	373	4058	0.14
ICC-7554	GSH	204	61	7.21	398	3908	0.16
ICC-6905	GSH	220	67	7.61	582	3414	0.15
ICC-2884	PSH	184	42	6.54	338	3918	0.18
ICC-9848	PSH	276	67	8.92	481	3676	0.16
ICC-3582	PSH	208	40	8.59	271	4296	0.13
ICC-2263	GSHN	263	59	7.12	404	4170	0.14
ICC-6306	GSHN	151	62	6.50	341	3268	0.14
ICC-11303	GSHN	297	111	6.38	406	4320	0.18
ICC-2969	GSHN	317	54	6.40	404	3443	0.16
ICC-15294	GSHN	153	50	7.54	311	3421	0.17
ICC-9002	PSHN	298	56	8.77	345	4116	0.15
ICC-9643	PSHN	328	117	7.95	437	4176	0.19
ICC-12492	PSHN	119	49	7.28	310	3044	0.18
ICC-13863	PSHN	138	65	7.91	450	4228	0.14
G-130	(Check)	229	41	6.21	271	3442	0.12
Mean		256	59	7.33	372	3848	0.16
SE±		28.8^{***}	2.3^{***}	0.232^{***}	21.9^{***}	216.4^{***}	0.004^{***}
CD (0.05)		83.4	6.8	0.672	63.4	627.9	0.012
CV (%)		16	6	5	8	8	4

SE = Standard error; LSD = Least significant differences; CV = Coefficient of variation; ***= statistically significant at 0.001; GN= Good nodulation; PN= Poor nodulation; GSH= Good shoot weight; PSH= Poor shoot weight; GSHN= Good shoot weight and good nodule number; GSHP= Poor shoot weight and poor nodule number

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