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Fast chlorophyll fluorescence transient and nitrogen fixing ability of chickpea nodulation variants

S.S. DUDEJA and P. CHAUDHARY

Department of Microbiology, CCS Haryana Agricultural University, Hisar 125004, India

Abstract

High nodulating (HN) selections of the cultivars ICC 4948 and ICC 5003 had the highest nodule number and nodule dry mass followed by low nodulating (LN) selections of the same cultivar. Both non-nodulating (NN) selections of cv. ICC 4993 and ICC 4918 did not show any nodule. Using N-difference method the HN selection of cv. 1CC 4948 was able to meet 73 % of its demand of N through biological fixation of N_2 [P(fix)], while 27 % of N demand was met by uptake from the soil, whereas its LN selection was able to meet only 54 % of its demand of N through biological fixation of N_2. Similarly in cv. ICC 5003 HN and LN selections the P(fix) was 76 and 64 %, respectively. Fast chlorophyll (Chl) fluorescence transient data analysis showed that performance index PI(abs) was 62.0 in cv. ICC 4948 HN selection and 44.5 in its respective LN selections. Corresponding values for cv. ICC 5003 were 32.4 and 28.4. In NN selections of ICC 4993 and ICC 4918 it was 12.6 and 30.7, respectively. Structure function index of the plants SFI(abs) and driving force for photosynthesis (DF) were highest in the HN selections followed by LN selections and lowest in the NN selections. The total uptake of N by chickpea plants was significantly and positively correlated with the density of reaction centres ABS/CS_0, TR_0/CS_0, and DI_0/CS_M, whereas total N uptake by chickpea seeds was significantly positively correlated with N and TR_0/CS_0. The percentage of P(fix) was highly significantly positively correlated with N, the so-called turnover number which indicates how many times Q_A has been reduced in the time span from 0 to t_{Fmax} and TR_0/CS_0. Fast Chl *a* fluorescence measurement can be used as a model system to assess the N fixation ability in chickpea.

Additional key words: *Cicer arietinum*; nitrogen uptake; P(fix).

Introduction

Nitrogen requirement of a crop is closely related to yield and in legumes N requirement is met by biological fixation of atmospheric N and partially N uptake from the soil. A legume dependent more on biologically fixed nitrogen, P(fix), is considered more beneficial than that dependent on uptake from the soil. Crop N status is also reflected in the leaves and is closely related to photosynthesis rate (Peng *et al.* 1995a,b). Changes in chlorophyll (Chl) content can occur as a result of nutrient deficiencies, exposure to environmental stresses and certain herbicides, and differences in irradiance during plant growth. Thereby Chl contents can be used to manage nutrient optimization programmes that both improve crop yield and help to protect the environment (Cassman *et al.* 1993, Peng *et al.* 1996). Relationship between the primary reactions of photosynthesis and Chl *a* fluorescence is also very useful and provides information about the photosynthetic capability and vitality of the plants (Lichtenthaler 1988, Genty *et al.* 1989, Schreiber *et al.* 1995, Owens 1996, Strasser *et al.* 1997). Therefore apart

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Fax: 91 1662 234952, e-mail: ssdudeja@hau.ernet.in
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Abbreviations: ABS, photons absorbed by the antenna pigment chlorophyll; CS, excited cross section of leaf; dV/dt_0 or M_0, slope at the origin of the fluorescence rise; DF_{Po}, total driving force for photosynthesis; DI_0, dissipated excitation energy at time zero; ET, electron transport flux; F_J, fluorescence intensity at the J step at 2 ms; F_0, F_M, initial and maximum Chl *a* fluorescence, respectively; F_V, variable fluorescence intensity; F_{150}, fluorescence intensity at 150 μs; F_{300}, fluorescence intensity at 300 μs; I_0, the incident photon flux; *N*, turn over number; PI, performance index; $PI_{Po,}$ linear distance between a point to the control; PS2, photosystem 2; Q_A, primary quinone acceptor of PS2; RC, reaction centre; SFI, structure-function-index; $SFI_{Po,}$ $SFI_{No,}$ subscripts "P" and "N" refer to photosynthesis and non-photosynthesis, respectively; S_M, normalized area; t_{Fmax}, time to reach the maximal fluorescence; TR, energy trapping flux; V_J, intermediate step; φ_{E0}, probability that an absorbed photon will move an electron into the electron transport chain beyond Q_A; φ_{P0}, maximum quantum yield of primary photochemistry; ψ_0, efficiency with which a trapped exciton can move an electron into the electron transport chain beyond Q_A.

from Chl contents, the fluorescent transient can be used to address the effects of herbicides, pesticides, hormones, nodulation, drought, heat, cold, and salt stress on crop productivity (Strasser *et al*. 1998, Schmitz *et al.* 2001).

To develop cultivars with better N_2 fixation ability and better crop productivity breeders must consider the underground features of a plant in the breeding programmes. Therefore there is a need to develop a non-destructive and rapid method for assessing the N_2 fixing potential of a cultivar. This is why the use of Chl *a* fluorescence measurement as a model system to assess the N_2 fixation ability in chickpea was explored. The fluorescent transient can be easily measured by handy plant efficiency analyzer (*PEA*). Further chickpea nodulation variants having variable nodulation and nitrogen fixation (high nodulating = HN; low nodulating = LN; non-nodulating = NN) were selected. These nodulation variants fix N in order of their nodulation ability (Rupela 1994, Dudeja *et al.* 1997, Sheoran *et al.* 1997). In the present study N fixation was monitored along with fast Chl fluorescence transient data in two nodulating variants of chickpea along with NN selections.

Materials and methods

Chickpea nodulation variants: HN and LN selections of chickpea (*Cicer arietinum* L.) cultivars ICC 4948 and ICC 5003 and two NN selections ICC 4918 NN and ICC 4993 NN were obtained from Dr O.P. Rupela, ICRISAT Asia Centre.

Field experiment was carried out at CCS Haryana Agricultural University Research Farm. Soil analysis of the fields showed that soil was sandy loam with soil pH (H_2O 1 : 2) 7.9; organic C (0.41 %); electrical conductivity (H_2O 1 : 2) 0.4 dS m^{-1}; total N 0.058 %, and phosphorus (Olsen) 11 g m^{-3}. The plants were uprooted after 45–50 d of plant growth for assessing the nodulation.

Chl *a* fluorescence was measured in field-grown plants after 90–100 d of growth. In each plot, 30 measurements were done. A compact, portable handy *PEA* analyser (*Hansatech*, UK) was used which works on continuous excitation fluorescence measurement principle. The fully expanded leaves were first acclimated to the dark for minimum 2 min by fixing clips. The dark adapted samples were continuously irradiated for 1 s (1 500 µmol m^{-2} s^{-1}) provided by an array of three light emitting diodes in the sensor. The fluorescence signals were detected using *PIN* photodiode after passing through a long pass filter (50 % transmission at 720 nm). The first reliable point of the fluorescence transient was measured at t_0 = 0.05 µs after the onset of irradiation and was taken as F_0. The data was analyzed using the software *Biolyser 4.0* programme developed and supplied free of cost by R. Maldonado Rodriguez (Bioenergetics Laboratory at the University of Geneva, Switzerland) using the JIP test (Strasser *et al.* 2000) which provides parameters indicating the efficiency of photosystem 2.

Total nitrogen content of the plants/seeds was estimated by digesting 0.2 g dried and ground plant material in di-acid followed by Kjeldahl's steam distillation method (Bremner 1965). A true measure of N_2 fixation was achieved by growing two NN and non-N_2 fixing chickpea cultivars ICC 4993 NN and ICC 4918 concurrently in the same soil. Nitrogen contents in plant, seed, and soil in all the nodulation variants and NN selections was used for calculating the proportion of N demand of chickpea being met by biological fixation of N_2 and being taken up from the soil using N-difference method (Herridge and Danso 1995). The difference in total N accumulated by N_2 fixing chickpea cultivars and non-N_2 fixing chickpea cv. ICC 4918 NN was regarded as the amount of N_2 fixed and P(fix) = N_2 fixed/N_2 fixed by N fixing chickpea.

Correlation of N_2 fixation and Chl *a* fluorescence: To assess which parameter of the O-J-I-P test of Chl fluorescence transient data is more closely related to N_2 fixation, statistical correlation coefficient within these different parameters was determined using Statistical Package for Social Sciences programme.

Results and discussion

The HN selections of cvs. ICC 4948 and ICC 5003 showed higher nodule number and nodule dry mass than the corresponding LN selections of the cultivars (Table 1). NN-selections of cvs. ICC 4993 and ICC 4918 did not show any nodules indicating that over the years the nodulation selections HN and LN were stable in their behaviour as reported earlier (Khurana and Dudeja 1996, Dudeja *et al.* 1997). But in NN selections ICC 4918 NN and ICC 4993 NN about 2–3 % reversion frequency was observed which could be attributed to spontaneous reversion, presence of some specific *Rhizobium* strain, and environmental conditions. The details of this are being investigated.

The N demand of chickpea being met by biologically fixed N, P(fix) was dependent upon the extent of nodulation. In the cv. 1CC 4948 the HN selection was able to meet 73 % of its demand of N through biological fixation of N_2, while 27 % of N demand was met by uptake from the soil, whereas its LN selection was able to meet only 54 % of its demand of N through biological fixation of N_2 (Table 1). Similarly in cv. ICC 5003 the HN selection was able to meet 76 % of its demand of N through biological fixation of N_2, while 24 % of N demand was met by uptake from the soil, whereas its LN selection was able to meet 64 % of its demand of N through biological fixation of N_2. Various legumes meet their requirement of N to different extents through biological N fixation. Peoples *et al.* (1995) reported that chickpea can fix 61–134 kg(N) ha^{-1} and 41–55 % of its N demand is derived through biological fixation of N, and rest is being taken up from the soil.

Fast Chl fluorescence kinetics of leaves of nodulation variants of both cvs. ICC 4948 and ICC 5003 was measured along with NN selections ICC 4993 and ICC 4918. Different yields of absorbed energy and specific fluxes at time zero were derived according to the JIP test (Strasser *et al*. 2000, Misra *et al.* 2001). Fast fluorescence transient (O-K-J-I-P curves) is shown in Fig. 1. The Chl *a* fluorescence was lowest in the NN cv. ICC 4993 followed by ICC 4918. The LN selection of cv. ICC 4948 showed lesser rise in Chl *a* fluorescence as compared to its HN selection. In nodulating variants of another cv. ICC 5003, a similar trend in Chl fluorescence rise was observed, but the rise was less than in the cv. ICC 4948. The NN selection ICC 4918 showed minimum Chl *a* fluorescence while the HN selections showed maximum Chl *a* fluorescence.

Using the *Biolyser* programme, a radar plot of fluorescence data with the NN selection ICC 4918 as control, different nodulation variants of both cultivars showed lower F_0/F_M values in HN and LN selections of ICC 4948 and ICC 5003 and another NN selection ICC 4993 (Fig. 2). All other parameters F_0, F_M, F_V/F_0, V_J, log S_m, N, phenomenological fluxes, and vitality indexes were greater in nodulation variants than in the NN selections.

Different mathematical expressions of O-J-I-P parameters considered in the present study are shown in Table 2. F_M (fluorescence at maximum) values of both LN selections were lower than in their respective HN selections. Phenomenological fluxes ABS/CS_0, TR_0/CS_0, ET_0/CS_0, and DI_0/CS_0 were comparatively greater in HN selections than in LN or NN selections except the ICC 4918 NN selection. Vitality or vigour of the chickpea plants like structure and function index of the plants SFI(abs) and performance index PI(abs) and driving force for photosynthesis (DF) were highest in the HN selections followed by LN selections, and lowest in the NN selections. The relative performance indexes and driving forces for energy fluxes were higher in HN selections and decreased

Table 1. Nitrogen fixation by chickpea nodulation variants grown under field conditions.

Nodulation variants	Nodules per plant	Nodule dry mass [mg per plant]	Nitrogen content [kg kg^{-1}] per shoot	seed	N demand met through biological N_2 fixation [P(fix)]
ICC 4993 NN	0	0	8.46	9.58	0
ICC 4918 NN	0	0	5.86	7.90	0
ICC 4948 HN	74	255	10.39	30.80	73
ICC 4948 LN	18	167	10.78	25.37	54
ICC 5003 HN	66	325	11.14	27.89	76
ICC 5003 LN	12	185	8.69	27.53	64

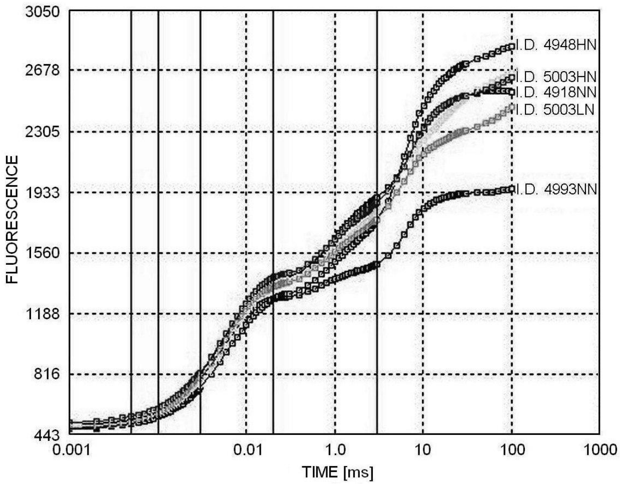

Fig. 1. Chlorophyll *a* fluorescence of the field grown HN and LN chickpea nodulation variants and non-nodulating lines.

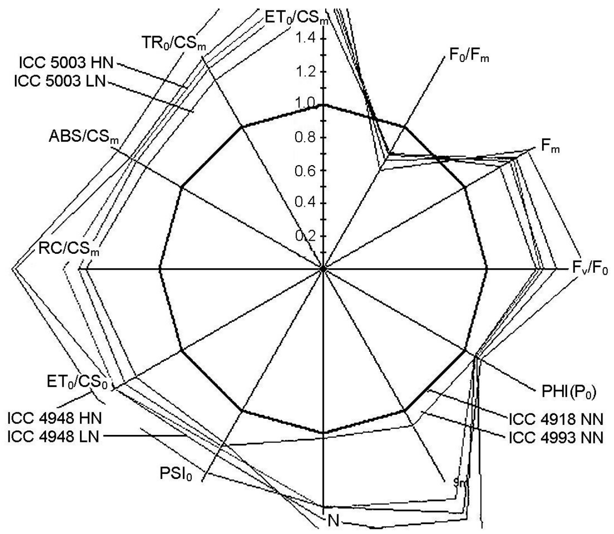

Fig. 2. Radar plot fluorescence data of different chickpea nodulation variants differing in extent of nodulation. The non-nodulating cv. ICC 4918 was taken as control.

Table 2. Quantum efficiency, relative performance index, and driving forces for energy fluxes of different chickpea nodulation variants differing in extent of nodulation.

Parameter	Ratios	Mathematical expressions	Chickpea nodulation variants ICC 4993 NN	ICC 4918 NN	ICC 4948 HN	ICC 4948 LN	ICC 5003 HN	ICC 5003 LN
Fluorescence parameters	F_M	ABS/CS_M	1945.0	2552.0	2810.0	2640.0	2613.0	2423.0
	F_0/F_M	$1 - \varphi_{Po}$	0.3	0.2	0.2	0.2	0.2	0.2
	F_V/F_0	TR_0/DI_0	2.8	3.7	4.4	3.9	3.6	3.6
	dV/dto or M_0	$4 (F_{300} - F_0)/(F_M - F_0)$	0.8	0.6	0.4	0.4	0.5	0.6
	V_j	$F_J - F_0/F_M - F_0$	0.5	0.4	0.3	0.4	0.4	0.4
	S_M	$Area/(F_M - F_0)$	32.5	35.8	55.8	63.1	52.5	57.3
	N	$S_m M_0 (1/V_J)$	45.8	47.4	66.1	69.7	66.7	73.8
Flux ratios per RC or specific fluxes	ABS/RC	$M_0 (1/V_J) (1/\varphi_{Po})$	1.91	1.68	1.45	1.38	1.62	1.65
	TR_0/RC	$M_0 (1/V_J)$	1.4	1.3	1.2	1.1	1.3	1.3
	ET_0/RC	$M_0 (1/V_J) \psi_0$	0.7	0.8	0.9	0.7	0.8	0.7
	DI_0/RC	$(ABS/RC) - (TR_0/RC)$	0.5	0.4	0.3	0.3	0.4	0.4
Flux ratios or quantum efficiencies	TR_0/ABS or φ_{P0}	$(1 - F_0)/F_M$ or F_V/F_M	0.7	0.8	0.8	0.8	0.8	0.8
	ET_0/ABS or φ_{Eo}	$(1 - F_0)/F_M \psi_0$	0.3	0.5	0.6	0.5	0.5	0.4
	ET_0/TR_0 or ψ_0	$1 - V_J$	0.5	0.6	0.7	0.6	0.6	0.6
Density of RCs	RC/CS_0	$\varphi_{Po} (V_J/M_0) F_0$	269.2	321.5	394.5	384.7	345.9	319.7
	RC/CS_M	$\varphi_{Po} (V_J/M_0) F_M$	1016.8	1522.4	1938.0	1909.2	1616.9	1472.5
Phenomenological fluxes or phenomenological activities	ABS/CS_0	F_0	515.0	539.0	544.0	532.0	559.0	526.0
	TR_0/CS_0	$\varphi_{Po} (ABS/CS_0)$	378.6	425.2	430.0	424.8	439.4	411.8
	ET_0/CS_0	$\varphi_{Po} \psi_0 (ABS/CS_0)$	176.1	246.3	280.4	258.4	258.2	232.5
	DI_0/CS_0	$(ABS/CS_0) (TR_0/CS_0)$	136.4	113.8	130.0	107.2	119.6	114.2
Vitality indexes	$SFI_{(ABS)}$	$(SFI_{Po})/(SFI_{No})$	1.8	2.7	3.8	3.5	2.9	2.7
	$PI_{(ABS)}$	$(RC/ABS) \varphi_{Po} \psi_0$	12.6	30.7	61.9	44.5	32.4	28.4
	$PI(CS_0)$	$ABS/CS_0\ TR_0/CS_0\ ET_0/CS_0$	6497.0	16528.0	31816.0	23663.0	18109.0	14949.0
	$PI(CS_M)$	$ABS/ CS_M\ TRo/CS_M\ ETo/CS_M$	24540.0	78257.0	173937.0	117429.0	84652.0	68862.0
Driving forces	D.F.	$\log (PI_{Po})$	2.5	3.4	4.1	3.8	3.5	3.3

with reduction in extent of nodulation and lowest in the NN selections. Performance index of the plants PI(abs) was 62.0 in HN selection of cv. ICC 4948 and 44.5 in its respective LN selections. Corresponding values for cv. ICC 5003 were 32.4 and 28.4. In the NN selections of ICC 4993 and ICC 4918 it was 12.6 and 30.7, respectively. Phenomenological leaf model of different nodulation variants of chickpea with activity of flux ratios is shown in Fig. 3. F_0/F_m, dV/dt_0, dVG/dt_0, and V_j values were higher in NN lines than in LN selections and were lowest in the HN selections. These ratios give rise to closed reaction centres. Phenomenological leaf model clearly showed that there were more inactive reaction centres (indicated by dark circles) in the NN lines of ICC 4918 and ICC 4948 in comparison to the chickpea nodulation variants. Among the LN and HN variants of both cultivars there were differences in inactive and active reaction centres. In LN selections more inactive reaction centres were present as compared to HN selections. The increased number of inactive reaction centres in Chl correspondingly results in decreased electron transport per excited cross section (ET_0/CS_0).

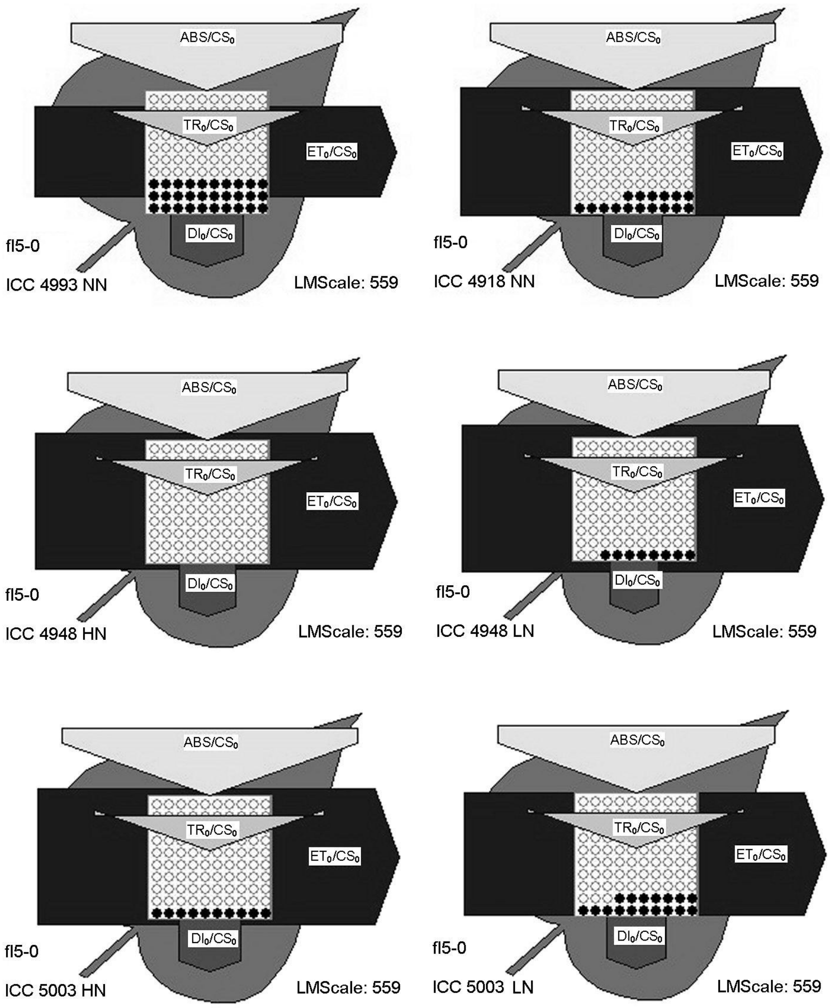

Fig. 3. Phenomenological leaf model (CS_0) of different chickpea nodulation variants differing in extent of nodulation and non-nodulating lines.

Nodulation was significantly positively correlated with ET_0/RC (Table 3). The total uptake of N by chickpea plants was significantly and positively correlated with the density of reaction centres ABS/CS_0, TR_0/CS_0, and DI_0/CS_M, whereas total N uptake by chickpea seeds was significantly positively correlated with N and TR_0/CS_0. Nitrogen demand of chickpea being met by P(fix) was significantly highly positively correlated with N, the so called turn over number which indicates how many times Q_A has been reduced in the time span from 0 to t_{Fmax} and TR_0/CS_0, the phenomenological activity, though the correlation with ET_0/CS_M, TR_0/CS_M, ABS/CS_M, ET_0/CS_o, and S_M was also significant. The N demand of chickpea plants being met by P(fix) was significantly negatively correlated with F_0/F_M, dv/dt_0, V_j, PHI(D_0), and DI_0/RC. The presence of inactive centres is the direct reflection of the nodulation status of the chickpea host. Schmitz *et al.* (2001) showed that DF that quantifies the potential of plant photosynthesis (Srivastava *et al*. 1999) as a function of different nitrate concentrations in correspondence with nodulation in *Vigna unguiculata* and JIP test are sensitive to N deficiencies and to the degree of nodulation by *Rhizobium.*

Table 3. Correlation between N_2 fixation and different chlorophyll fluorescence transient parameters of chickpea.

S. No.	Fluorescence transient parameters	Nodule No.	Mg(N) plant^{-1}	Mg(N) seed^{-1}	P(fix) [%]
1.	F_0/F_M	-0.695	-0.509	-0.592	-0.854
2.	F_V/F_0	0.713	0.443	0.508	0.789
3.	dv/dt_0	-0.654	-0.480	-0.525	-0.824
4.	V_j	-0.757	-0.518	-0.558	-0.825
5.	V_i	-0.730	-0.272	-0.252	-0.568
6.	$PHI(P_0)$	0.695	0.509	0.592	0.854
7.	PSI_0	0.757	0.518	0.558	0.825
8.	$PHI(E_0)$	0.748	0.503	0.548	0.818
9.	$PHI(D_0)$	-0.695	-0.509	-0.592	-0.854
10.	S_m	0.342	0.457	0.646	0.865
11.	*N*	0.332	0.558	0.819	0.913
12.	ABS/RC	-0.492	-0.381	-0.434	-0.763
13.	TR_0/RC	-0.377	-0.281	-0.311	-0.666
14.	Et_0/RC	0.871	0.632	0.682	0.708
15.	DI_0/RC	-0.609	-0.485	-0.562	-0.849
16.	RC/CS_0	0.498	0.498	0.484	0.788
17.	ABS/CS_0	0.347	0.843	0.664	0.552
18.	TR_0/CS_o	0.700	0.887	0.829	0.914
19.	ET_0/CS_0	0.786	0.657	0.661	0.898
20.	DI_0/CS_0	-0.607	-0.298	-0.424	-0.717
21.	RC/CS_m	0.627	0.461	0.478	0.796
22.	ABS/CS_m	0.768	0.639	0.654	0.896
23.	TR_0/CS_m	0.760	0.602	0.627	0.879
24.	ET_0/CS_m	0.773	0.536	0.556	0.824
25.	DI_o/CS_m	0.345	0.843	0.664	0.551
26.	SFI(abs)	0.632	0.393	0.426	0.753
27.	10RC/ABS	0.445	0.303	0.340	0.694
28.	$PHI_0/(1 - PH)$	0.713	0.443	-0.592	0.789
29.	$PSI_0/(1 - PS)$	0.774	0.428	0.445	0.727
30.	PI(abs)	0.704	0.320	0.334	0.654
31.	$PI(CS_0)$	0.724	0.372	0.373	0.690
32.	$PI(CS_m)$	0.723	0.328	0.328	0.646

Chickpea was able to meet 54–76 % of its demand of N through biological fixation of N_2, while remaining demand of N was met by an uptake from the soil, so there is sufficient scope for improvement as legumes meet up to 95 % of the demand through biological fixation of N_2. Fast Chl fluorescence transient data analysis showed that PI(abs), SFI(abs), and DF could directly be used for monitoring the nodulation status. Total uptake of N by chickpea plants was significantly and positively correlated with the density of reaction centres indicating that the fast Chl *a* fluorescence measurement could be used as a model system to assess the N fixation ability of chickpea. This will help in the development of better N_2 fixing cultivars by the breeders.

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