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Evaluation of Physiological and Biochemical Contents in Desi and Kabuli Chickpea

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ABSTRACT

Background: Chickpea (*Cicer arietinum* L.), a self-pollinating legume being cultivated globally as a rich source of vegetarian protein. It plays an important role in human feed and nutritional security, especially in agricultural-based communities. Chickpea has higher bioavailability of protein, good sources of polyphenols and flavonoids. Besides their nutritional value, chickpea seeds contain various phenolic compounds. Phenolic compounds are of particular interest due to their contribution to the seed colour, sensory characteristics and several biological properties. Flavonoids are one of the main groups of phenolic compounds found in grain legumes. Desi and Kabuli chickpeas are being used worldwide and there are few studies where both desi and kabuli chickpeas seed flour for above properties with respect to physiological traits has been reported.

Methods: The present investigation has been formulated to compare popular chickpea genotypes of desi and kabuli types for biochemical parameters viz., protein content, amino acid, total flavonoid content, total phenolic contents and RSA as well as two physiological traits i.e., chlorophyll content and leaf area index. The experiment consisted of 44 genotypes grown in Randomized Block Design with row to row distance of 30 cm, in two replications during *Rabi* 2018-19.

Result: The average crude protein content in desi and kabuli chana varied from 18.2% (Dollar variety) to 26.7% (JG315) and total phenolic content (TPC) ranged 1.22 to 0.74 mg/g. Total Flavonoid content (TFC) varied from 0.39 (ICCV-2) to 0.61 mg/g (JAKI-9218) with mean value of 0.47. Radical scavenging activity (RSA) in chickpea genotypes ranged from 36.2 to 49.5% with mean value of 40.86%. Total amino acid significantly correlated with TPC and TFC and TPC significantly correlated with TFC at 5% significant level.

Key words: Desi Chana, DPPH, Kabuli Chickpea, Leaf area index

INTRODUCTION

Chickpea (*Cicer arietinum* L.), a self-pollinating diploid (2n=2x=16) species with a genome size of 740 Mbp, is the world's second largest grown pulse crop after beans. Chickpea is a valuable legume due to rich source of protein. India is the producer of 75% of world's chickpea production. Production of chickpea has increased worldwide by 56% and during in period 2004–2013 in India by 55%. Australia, Pakistan, Myanmar, Ethiopia, Mexico, Canada, USA, Tanzania and Malawi are other chickpea producing countries (Gaur *et al*. 2016). The United Nations General Assembly declared the year 2016 as International year of pulses with the record production of pulses in India of 17.56 Mt. Also, India emerged as the largest chickpea producer in the world with the production of 7.8 Mt (Kumar *et al*.2018; Kaur *et al*. 2019).In India, the major chickpea producing states are Madhya Pradesh, Uttar Pradesh, Rajasthan and Maharashtra. Madhya Pradesh is the largest producer of chickpea in the country accounting for over 40 per cent of the total national production.

Pulses have both environmental and nutritional benefits, they are often recommended in sustainable diets (Chaudhary *et al*. 2018). Their environmental benefit is related to their ability to restore soil nitrogen by process of nitrogen fixation. The Food and Agriculture Organization (FAO) recommend pulses as staple food to fulfil the basic protein and energy requirements of the human diet (Mudrai *et al*. 2014). Due to the balanced nutrient composition, chickpea is a popular pulse in human diet. Chickpea is an outstanding source of nutritional constituents such as proteins and minerals (Gupta *et al*. 2017).Cereals are rich in thiol containing amino acids (methionine and cysteine) and deficient in lysine whereas pulses are rich in lysine and deficient in methionine and cysteine. In human diet proper intake of essential amino acids, pulses are taken with addition of cereals (Reinkensmeier *et al*. 2015). Indeed, pulse grains are a low-fat source of proteins and carbohydrates. Now a day's pulses have main interest as a gluten-free food category. They exhibit complementary amino acid profiles to those of cereals in well-balanced semi-

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vegetarian or plant-based diets. They are also rich in fibres and contain variable amounts of other nutritional components such as vitamins, minerals and bioactivities. This is likely associated with improved risk of developing pathologies such as diabetes, cardiovascular or degenerative diseases (Gupta *et al*. 2019).

Chickpea are categorized into desi and kabuli types. The desi (microsperma) types have pink flowers, anthocyanin pigmentation on stems and seed coat is thick and coloured. The Kabuli (macrosperma) types have white flowers, stems deficient of anthocyanin pigmentation, seeds are white or beige coloured and a smooth surface with thin seed coat. Geographical distribution of these chickpea types distinctly separate them as desi types which are mostly grown in Asia and Africa and account for up to 80–85% of the total chickpea area and kabuli chickpea types are grown mostly in West Asia, North Africa, North America and Europe (Gaur *et al*. 2016).

Natural antioxidants are considered secure for the consumers than synthetic antioxidants such as butylated hydroytoluene (BHT), which have carcinogenic effects. Desi and kabuli chickpea significantly differ at nutritional level due to variations with respect to physiochemical properties, protein digestibility, phenolic content and antioxidant activity (Heiras-Palazuelos *et al*. 2013). Previous studies have shown that kabuli seeds are more nutritive in respect to protein content (Purushothaman *et al*., 2014), however, desi chickpeas are known for higher antioxidant activity (Segev *et al*. 2011). Macar *et al*.(2017)in a comparative study on total phenolic content (TPC), total flavonoid content (TFC) and total protein content among desi and Turkish kabuli chickpea genotypes found that Turkish genotypes are rich in protein level, but desi type are valuable for its high total phenolic and flavonoid contents. Other studies concluded that the dark-colored and pigmented pulses (including chickpea) tend to have higher phenolic content compared to the light coloured varieties and the content of phenolics change due to storage and processing (Parmar *et al*. 2017; Segev *et al*., 2010; Singh, 2017) of the grains. The chickpea seeds with dark color exhibited higher levels of antioxidant activity, making them more acceptable as functional foods (Segev *et al.* 2010; Singh, 2017). Photosynthetic pigments and leaf area index plays important role in growth and development of all crops. Leaf area is an important observation dealing with photosynthetic efficiency, light interception, evapotranspiration, plant growth and response toward fertilizers and irrigation (Blanco and Folegatti 2005).

Chickpea is the second largest cultivated legume globally as a rich and cheap source of vegetarian protein, which plays an important role in human feed and nutritional security in most low income agricultural-based communities. Among the different types of grain legumes, chickpea has been reported to have a higher bioavailability of protein constitutes about 80% of the total dry seed mass (Jukanti *et al* 2012; Yust *et al*. 2003). Dry legumes are good sources of polyphenols and flavonoids. Besides their nutritional value, chickpea seeds contain various phenolic compounds. Phenolic compounds are of particular interest due to their contribution to the seed colour, sensory characteristics and several biological properties (Magalhães *et al*. 2017). Flavonoids are one of the main groups of phenolic compounds found in grain legumes (Magalhães *et al*. 2017). Desi and Kabuli chickpeas are being used worldwide and there are few studies where both desi and kabuli chickpeas seed flour for above properties with respect to physiological traits has been reported. Therefore, the present investigation has been formulated to compare popular chickpea genotypes of desi and kabuli types for biochemical parameters *viz*., protein content, amino acid, total flavonoid content, total phenolic contents and RSA as well as two physiological traits i.e., chlorophyll content and leaf area index. The identified genotypes with superior seed quality, biochemical and physiological parameters can be further utilized in hybridization programme for varietal improvement.

MATERIALS AND METHODS

Plant material

Plant material consisted of forty four chickpea genotypes received from RAK Krishi College, Sehore, Madhya Pradesh. The genotypes included both types of chickpea desi *i.e*., JG-63, RVG-202, JGG-1, DINDORI-CHANA, BHUPDA-CHANA, JG-322, GCP-101, JG-11, RVG-203, ANNAGIRI, JG-16, JG-14, JG-6, ICCV-10, VIJAY, JG-218, RSG-888, RVG-201, ICC-4812, JAKI-9218, JG-315, GG-5, JG-74, JG-12, RVSSG-205, RVSSG-204, GBM-2, JG-62 and kabuli chana (15) *i.e*., KRIPA, DOLLAR, PKV-4, ICCV-2, JGK-2, BGD-128, KAK-2, JGK-5, RVKG-102, RVKG-101, JGK-1, RVSSG-30, RVSSG-37, RVSSG-24 and MNK-1.

The experiment was carried at breeding farm, College of Agriculture, Gwalior (Madhya Pradesh). The experimental area occupied was quite uniform in respect of topography and fertility. Gwalior is situated at 22043' N Latitude and 760 54 E longitudes and altitude 618 m at above the mean sea level. This region has subtropical, semi- arid climate with hot and dry summers and cold winters with occasional showers. The average rainfall is about 23 mm (October to December, 2018) and 27 mm (January to March, 2018). The experiment consisted of 44 genotypes grown in Randomized Block Design with row to row distance of 30 cm, in two replications during *Rabi* 2018-19. Fertilizer was applied in the ratio of 20 N: 40 P_2O_5: 20 K_2O kg/ha. Observations were recorded as per the standard DUS guidelines for chickpea. For evaluation of yield and yield contributing traits, five random plants were selected from each line in every replication.

Physiological parameters

Chlorophyll content of chickpea genotypes

Photosynthetic pigments were estimated by Arnon's method (1949). Leaf samples of chickpea genotypes were collected after 60 days of sowing. Samples were crushed with mortar and pestles in liquid nitrogen to avoid the chemical

degradation of the chlorophyll (Fig 3). Resultant 100 mg leaf powder was added to 10 ml of acetone (80%) in 15 ml centrifuge tubes and cooled at 4°C for 15 min followed by centrifugation for 10 min at 10000 rpm before transferring the supernatant to fresh 15 ml centrifuge tubes. Quantification of photosynthetic pigment was performed using UV-VIS spectrophotometer for recording absorbance at 645 and 663 nm and 470 nm using 80% acetone as a blank. The amount of chlorophyll a, chlorophyll b and total chlorophyll were calculated according to Arnon's equation (1949).

Chlorophyll a = 12.21 OD663 – 2.81 OD645;
Chlorophyll b = 20.13 OD645 – 5.03 OD663;
Total Chlorophyll = Chlorophyll a + Chlorophyll b;

Leaf area analysis

The leaf length, leaf width leaf area index and leaf perimeter of all 44 chickpea genotypes were recorded at 30 and 60 days after sowing by using Biovis Leaf Av Instruments. Five random plants were considered to take leaf samples for each genotypes.

Estimation of Biochemical parameters of chickpea genotypes

Extraction and estimation of total protein and total amino acid content

Hundred milligram of seed powder was vortexed using 1 ml of 100 mM Tris–HCl buffe (pH 8). The resulting homogenates were centrifuged at 10,000 rpm for 20 min at cooling conditions and supernatants were collected. The total seed protein content supernatant was determined by the method of Bradford (1976) using bovine serum albumin (BSA) as a positive control for preparing the calibration curve. Five hundred milligram of defatted seed powder 5 to 10 ml of 80% ethanol was added following methods of Moore and Stein. The homogenate so obtained was centrifuged. Extraction was repeated thrice with the supernatants collected each time and pooled. These were then used as a source for estimation of total free amino acids. Leucine was used as a positive control. The intensity of the sample was read at 570 nm using UV spectrophotometer.

Estimation of total phenolic content (TPC) and total flavonoid content (TFC)

A modified Folin–Ciocalteu (FC) colorimetric method of Swain and Hills (1959) was used to estimate TPC. Seed homogenates were centrifuged, supernatant vacuum dried and finally taken up in distilled water. From this, TPC estimation was performed at 650 nm against a reagent blank and gallic acid used as a positive control at series concentrations. TFC of methanolic extract was estimated following Khoo *et al.* (2013). One millilitre from each seed extracts was added to 1 ml of 2% aluminium chloride ($AlCl_3$), incubated for 10 min and absorbance measured at 415 nm using a calibration curve made from quercetin (as a positive control) with methanolic AlCl as blank.

Antioxidant activity DPPH radical-scavenging activity

Radical scavenging activity (RSA) of 2, 2-diphenyl-1-picrylhydrazyl (DPPH) was determined using the method of Bondet *et al.* (1997). One millilitre of methanolic seed extract (50 mg in 1 ml methanol) was added in 3 ml of 0.1 mM DPPH. After vigorous mixing, it was allowed to stand in dark at room temperature for 30 min and its absorbance was measured at 517 nm and ascorbic acid was used as a positive control. The radical scavenging activity was calculated by the relation:

(A blank- A sample)/A blank) × 100 A is absorbance at 517 nm.

Characterization of chickpea proteins

Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE)

Protein profiling of chickpea genotypes was done by using SDS-PAGE as per method reported by Laemmli (1970). Chickpea protein seed were ground to make fine powder and preserved in air-tight plastic containers at room temperature until they were used. Around 25 mg seed powder was dissolve in 1 ml sample buffer (distilled water, 0.5 M Tri-HCl pH 6.8), Briefly vortexed for 30 mins at 5000 rpm and centrifuged for 30 mins at 5000 rpm. Take protein as supernatant in other tube. Then 10-20 ul protein sample and 2x protein dye (SDS, glycerol, bromophenol blue, DTT), mix properly and kept in boiling water (heated at 98 ºC) for 5-10 minutes, then loaded in 12% running gel and 5% staking gel in vertical electrophoresis unit at 100V for 1.5 to 2 h. The standard protein marker (PageRuler™ Plus Prestained Protein Ladder) which contained 10 to 250 kDa, was loaded to be used as standard for molecular weight estimation. The gels were visualized using Staining solution (Coomassie Brilliant Blue R-250) for 1-2 hr or overnight. The stained gels were de stained by changing the fixing solution 1:4:5 (glacial acetic acid, distilled water, methanol) until the excess stain disappeared.

Statistical analysis

To determine significant differences among all the genotypes the one way analysis of variance followed by post hoc analysis was applied by NTSYS pc 2.02 (Rohelf, 2000). The multivariate principal component analysis was applied to cluster the genotypes with XLSTAT 2013 software.

RESULTS AND DISCUSSION

Physiological parameters

Leaf area index (LAI) correlation analysis

Leaf area index was measured at 30 and 60 days after sowing. The mean value of leaf area after 30 days of sowing was 1.04 cm^2, perimeter 12.24 cm^2, leaf length 4.18 cm^2 and leaf width 2.21 cm^2. The maximum range of leaf area was 2.0 cm^2, perimeter 21 cm, leaf length 6 cm and leaf width 5 cm. The mean value after 60 days of sowing for leaf area was recorded 2.56 cm^2, perimeter 6.57 cm, length 2.59 cm and width 1.23 cm and the maximum value of area was 4 cm^2, perimeter 11 cm, length 5 cm and width 2 cm

Table 1: Descriptive statistics of leaf area (LA), length (LL), width (LW) and perimeter (P) observed in chickpea genotypes leaves at 30 and 60 days, respectively.

	LA-30	LL-30	LW-30	P-30	Ratio L-W-30	LA-60	LL-60	LW-60	P-60	Ratio L-W-60
Mean	1.04	4.18	2.21	12.24	4.95	2.56	2.59	1.23	6.57	5.14
Std. Error of Mean	0.060	0.163	0.117	0.624	0.219	0.094	0.101	0.045	0.245	0.160
Std. Deviation	0.400	1.081	0.779	4.140	1.452	0.623	0.670	0.297	1.623	1.063
Maximum	2	6	5	21	10	4	5	2	11	8

*All values are presented in cm^2.

*LA30= Leaf area at 30 days; LL 30= leaf length at 30 days; LW30=leaf width at 30 days; P 30 =periphery at 30 days; ratio L-W 30= ratio of leaf length and width at 30 days; LA60= Leaf area at 60 days; LL 60= leaf length at 60 days; LW60=leaf width at 60 days; P 60=periphery at 60 days; ratio L-W 60= ratio of leaf length and width at 60 days

Table 2: Significance of Correlation of leaf area index at 30 and 60 days of chickpea genotypes analysed.

	LA_30	P_30	LL_30	LW_30	Ratio_L_W_30	LA_60	P_60	LL_60	LW_60	Ratio_L_W_60
LA_30	1	-.242	-.237	-.081	-.123	-.128	.756**	0.723**	0.733**	-.034
P_30		1	0.765**	0.411**	0.179	0.499**	-.189	-.220	-.093	-.052
LL_30			1	0.547**	0.407**	0.344*	-.052	-.080	0.032	-.018
LW_30				1	-.335*	0.023	0.223	0.183	0.071	0.094
Ratio_L_W_30					1	0.198	-.222	-.218	0.050	-0.198
LA_60						1	-.341*	-.353*	-.006	-.301*
P_60							1	0.957**	0.738**	0.239
LL_60								1	0.582**	0.448**
LW_60									1	-.399**
Ratio_L_W_60										1

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

*LA30= Leaf area at 30 days; LL 30= leaf length at 30 days; LW30=leaf width at 30 days; P 30 =periphery at 30 days; ratio L-W 30= ratio of leaf length and width at 30 days; LA60= Leaf area at 60 days; LL 60= leaf length at 60 days; LW60=leaf width at 60 days; P 60=periphery at 60 days; ratio L-W 60= ratio of leaf length and width at 60 days

Table 3: Cluster group for leaf area index of chickpea genotypes.

Cluster Number	Total Genotypes	Name of Genotypes
Cluster 1	6	JG-63, RVG-201, RVG-203, JG-16, ANNAGIRI, JGG-1
Cluster 2	20	DINDORI CHANA, BHUPDA CHANA, JG-322, GCP-101, JG-1, MNK-1, RVKG-101, JG-16, JGK-1, RVSSG-30, ICCV-2, VIJAY, GBM-2, RVSSG-24, PKV-4, GG-5, RVSSG-204, KRIPA, RVSSG-37, JG-6
Cluster 3	18	JG-14, RVG-201, ICCV-10, RSG-888, JG-12, JG62, JG-6, JG-218, JG-74, ICC-4812, DOLLAR, BGD-128, JG-315, RVSSG-205, RVKG-102, JAKI-9218, KAK-2, JGK-5.

*Cluster analysis done by using NTSYS ver 2.0 software.

(Table 1). Leaf area taken at 30 days was positively significantly correlated with leaf length of 60 days (r=0.723) and leaf width of 60 days (r=0.733) at p=0.01 level. The perimeter of 30 days is highly significant and correlated with length 30 (r=0.765), LW 30 (r=0.411) and leaf area 60 (r=0.499) at 0.01 level. Leaf length of 30 days was highly significant with leaf width of 30 days (r=0.547) at 1% significant level. Leaf area of 60 days showed negative correlation with leaf perimeter and leaf length. Leaf Perimeter was significantly correlated with LL60 (r=0.957) and LW60 (r=0.738) and leaf length was positively correlated with LW 60 (r=0.582) at 1% significant level (Table 2). Cluster analysis of leaf area index for chickpea genotypes done using NTSYS ver 2.0 software. Total 3 clusters were formed *i.e.,* cluster 1 having 6 genotypes, cluster 2 having 20 genotypes and cluster 3 with eighteen genotypes. Cluster I represented all desi chickpea including JG63, RVG201, RVG203, JG16, Annagiri and JGG 1 based

on value of leaf area index at 30 and 60 days after sowing (Table 3). All other clusters were having both desi and kabuli genotypes of chickpea.

Chlorophyll analysis and Correlation coefficient with leaf area index

Chlorophyll a, b and total chlorophyll of chickpea leaves at 60 days was recorded with UV spectrophotometer at 645, 663, 470 wavelength. The mean value for chlorophyll a= 1.66, chlorophyll b= 0.51 and the total chlorophyll =2.17was recorded. The range of chlorophyll a was 1 to 2, chlorophyll b was 0 to 1 and total chlorophyll of 1 to 3 (Table 4). The total chlorophyll was showing highly significant and positive correlation with Chl a and Chl b at 1% significance level. There was no positive correlation between leaf area index with chlorophyll content although leaf area at 60 days showed negative correlation with Chl b and total chlorophyll at 1% and 5% significant level, respectively (Table 5). Comparative diagram of leaf area at 30 and 60 days and chlorophyll content is presented in Fig 1.

Biochemical analysis

All the forty four genotypes of chickpea were used for analysis of total protein, total amino acid, total phenolic content (TPC) and total flavonoid content (TFC), DPPH and radical-scavenging activity (RSA).

Table 4: Descriptive statistics of Chlorophyll content and leaf area for chickpea genotypes.

	Chl_a	Chl_b	Chl_total	LA_30	LA_60
Mean	1.66	.51	2.17	1.04	2.56
Std. Error of Mean	.030	.026	.041	.060	.094
Median	1.66	.46	2.15	1.02	2.48
Minimum	1	0	1	0	1
Maximum	2	1	3	2	4

*Chl a=Chlorophyll a; Chl b= Chlorophyll b; Chl total = Total chlorophyll; LA30; leaf area at 30 days; LA60=Leaf area at 60 days

Table 5: Significance of Correlation of leaf area index and chlorophyll content of chickpea genotypes.

	Chl_a	Chl_b	Chl_total	LA_30	LA_60
Chl_a	1				
Chl_b	0.051	1			
Chl_total	0.778**	0.668**	1		
LA_30	0.046	0.215	0.170	1	
LA_60	-.116	-.399**	-0.338*	-0.128	1

**. Correlation is significant at the 0.01 level (2-tailed).*. Correlation is significant at the 0.05 level (2-tailed).

*Chl a=Chlorophyll a; Chl b= Chlorophyll b; Chl total = Total chlorophyll; LA30; leaf area at 30 days; LA60=Leaf area at 60 days

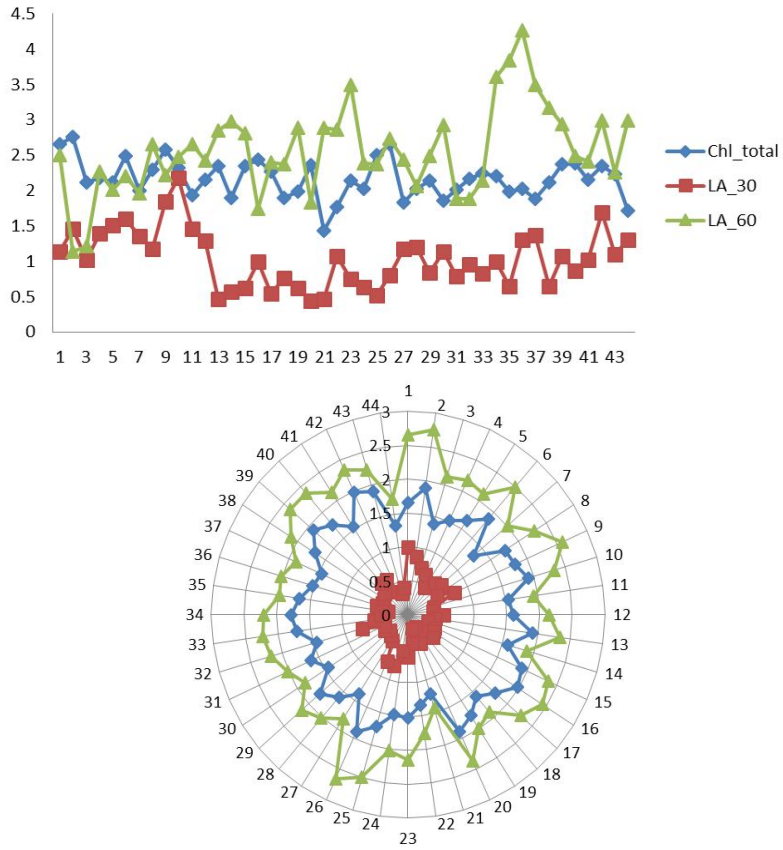

Fig 1: Comparative line diagram of leaf area index and chlorophyll content of chickpea genotypes. (Green line showing total chlorophyll contents, blue line leaf area at 30 days, brown line leaf area at 60 days after sowing).

Table 6: Total protein, amino acid, TPC, TFC and RSA values of chickpea genotypes.

Genotypes	Protein	Total amino acid	TPC	TFC	RSA
RVSKG-102	22	5.7± 0.8	0.88± 0.9	0.46± 1.1	41.4± 1.2
JG-62	23.2	5.9± 0.5	0.98± 0.9	0.44± 1.2	39.2± 1.5
RVSSG-205	24.6	6.1± 0.7	1.10± 1.1	0.57± 1.1	42.4± 1.4
JG-315	26.7	6.8± 0.7	0.97± 1.2	0.51± 0.8	44.6± 1.2
BHUPDA CHANA	19.3	4.1± 1.4	0.74± 1.3	0.41± 0.6	38.6± 1.0
ANNAGIRI	22.2	5.3± 1.3	0.90± 1.4	0.50± 0.6	39.2± 1.1
RVSSG-204	22.3	5.4± 1.2	0.92± 1.6	0.51± 0.8	38.7± 1.3
DINDORI CHANA	22.4	5.5± 1.4	0.96± 1.7	0.53± 1.1	36.2± 1.6
BGD-128	20.9	4.7± 1.5	0.82± 1.4	0.40± 1.7	38.5± 0.8
ICCV-2	21.2	4.9± 1.6	0.77± 1.3	0.39± 1.6	39.5± 0.9
RVSSG-37	22.4	5.3± 1.7	0.81± 1.4	0.41± 1.7	41.2± 0.5
KRIPA	19.6	4.3± 1.8	0.77± 1.1	0.40± 1.8	38.9± 0.6
DOLLAR	18.2	3.8± 1.8	0.79± 1.2	0.41± 1.1	36.4± 0.5
KAK-2	21.5	5.1± 1.1	0.92± 1.3	0.52± 1.2	40.1± 1.7
JG-36	22.6	5.7± 1.4	0.96± 1.4	0.54± 1.3	42.8± 1.7
RVSSG-24	19.8	4.5± 1.6	0.78± 1.1	0.44± 1.4	38.9± 1.8
PKV-4	18.6	4.0± 1.7	0.78± 1.2	0.46± 1.5	37.2± 1.5
JG-63	21.8	5.2± 0.9	0.89± 1.2	0.49± 1.6	38.4± 1.5
RVSSG-30	19.4	4.2± 0.8	0.91± 1.1	0.50± 0.6	39.1± 1.4
RVKG-101	21.2	4.9± 0.7	0.94± 0.2	0.52± 0.6	40.1± 1.2
ICC-4812	19.8	4.4± 0.6	0.76± 0.2	0.42± 0.6	38.7± 1.4
MNK-1	18.8	4.1± 0.6	0.86± 0.2	0.40± 0.7	39.1± 1.1
JGK-1	22.6	5.6± 0.5	0.91± 0.4	0.47± 0.8	39.2± 1.4
JGK-5	22.4	5.5± 1.1	0.97± 0.7	0.50± 0.9	40.1± 1.1
GCP-101	19.8	4.4± 1.2	0.87± 0.4	0.41± 1.1	36.7± 1.2
JG-218	21.1	5.0± 1.4	0.86± 0.7	0.40± 1.4	39.2± 1.1
JG-16	20.7	4.6± 0.9	0.88± 0.7	0.39± 1.5	38.9± 1.5
RVG-201	21.4	5.1± 1.1	0.85± 0.5	0.54± 1.4	41.2± 1.4
RVG-202	23.6	6.0± 1.2	0.96± 0.6	0.58± 0.5	42.6± 1.2
JAKI-9218	24.2	6.1± 1.2	1.22±0.8	0.61± 0.6	42.2± 1.3
JGG-1	22.8	5.7± 1.3	1.14± 0.9	0.56± 0.7	40.2± 1.2
RVSSG-205	20.6	4.6± 1.4	0.98± 0.7	0.41± 0.7	38.9± 0.8
JG-322	21.5	5.0± 1.6	0.96± 0.6	0.48± 0.9	41.2± 1.2
GBM-2	19.8	4.2± 1.7	0.74± 0.1	0.41± 1.1	40.4± 1.2
RSG-888	21.2	5.0± 1.8	0.87± 0.2	0.44± 1.4	45.6± 0.7
JG-12	21.7	5.2± 1.7	0.87± 0.1	0.46± 1.2	47.2± 0.6
JG-74	21.8	5.2± 1.4	0.89± 0.1	0.51± 1.1	48.1 ± 0.4
JG-14	21.9	5.2± 1.6	0.91± 0.1	0.52± 1.1	49.5 ± 1.1
JG-6	20.7	4.6± 1.7	0.78± 0.4	0.45± 0.8	45.4 ± 1.2
GG-5	19.6	4.1± 1.6	0.79± 0.3	0.46± 0.9	40.5 ± 1.3
RVG-203	20.3	4.3± 1.7	0.84± 0.3	0.50± 0.7	41.3 ± 1.5
JG-11	22.8	5.4± 1.1	0.91± 0.4	0.54± 0.6	43.2 ± 1.4
VIJAY	23.4	5.8± 1.2	0.99± 0.2	0.57± 0.8	45.7 ± 1.5
JGK-2	21.7	5.2± 1.3	0.87± 0.3	0.42± 0.7	41.7 ± 1.6
Avg.	21.4	5.0	0.89	0.47	40.8 ± 1.5
Max.	26.7	6.8	1.22	0.61	49.5 ± 1.8
Min.	18.2	3.8	0.74	0..39	36.2± 1.9

* Protein, Total amino acid, Total Phenolic Contents (TPC), Total flavonoid content (TFC) and radical-scavenging activity (RSA) represented in %, mg/g, mg/g, mg/g and % respectively.

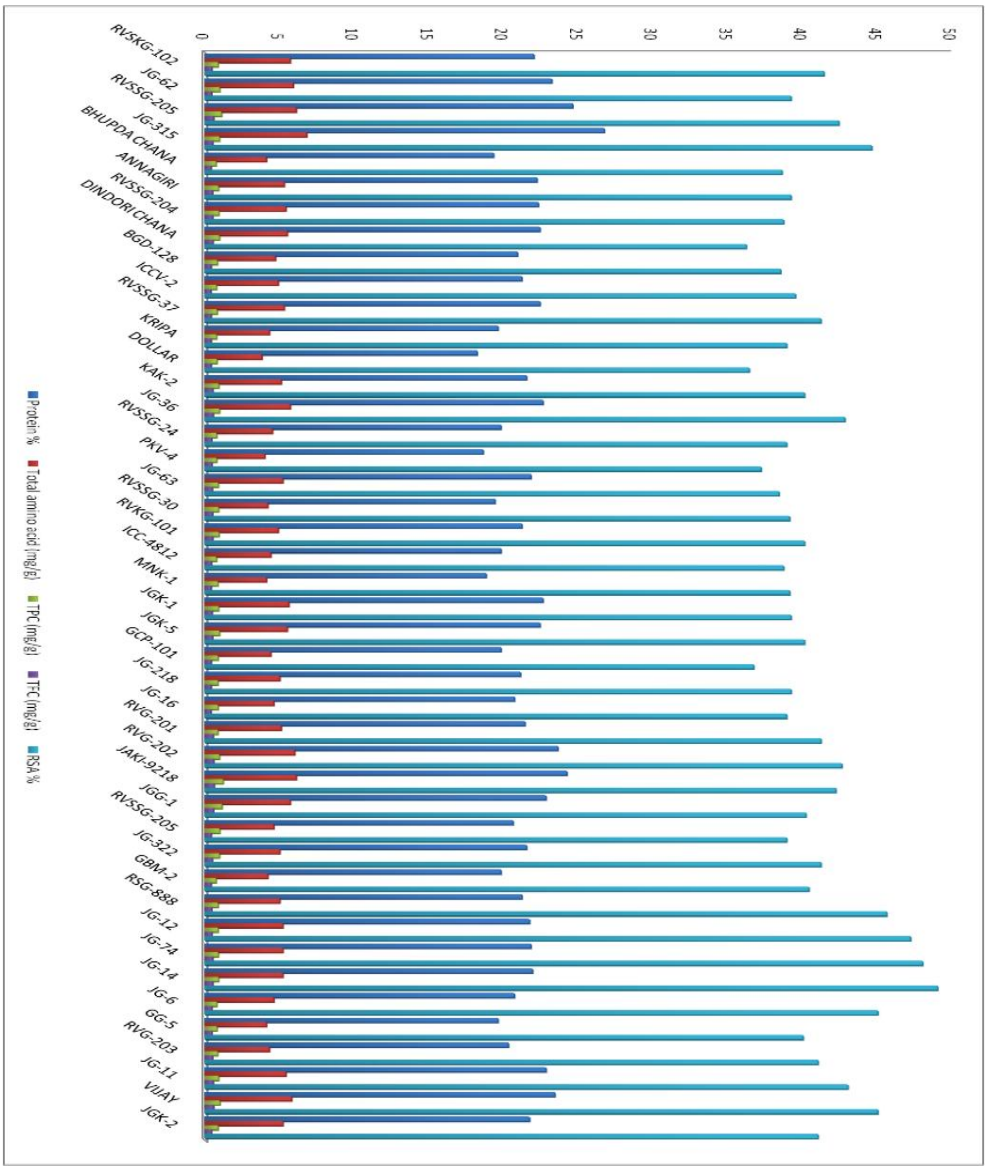

Fig 2: Bar diagram showing comparative range values of protein, total amino acid, Total phenolic contents, total flavonoid content and Radical scavenging activity for 44 chickpea genotypes.

Total protein and total amino acid content

Legumes are known as 'meat of the poor people' because of their high protein content and are considered as staple food for those who cannot afford animal proteins or vegetarian by choice and for people affected by nutrition related health problems such as diabetes, obesity and overweight. Chickpea has protein quality better than other legumes and is a good source of dietary protein (Gupta *et al.*, 2017). The crude protein content in chickpea genotypes (Table 6) ranged from 18.2% (Dollar variety) to 26.7% (JG315) with the mean value of 21.4%. Singh *et al.,* (2010) reported that protein content in chickpea genotypes ranged between 15.7 and 31.5% (Fig 2). Total Amino acid from 3.8± 1.8mg/g (Dollar variety) to 6.8± 0.7 mg/g (JG315).

Total phenolic content (TPC) and total flavonoid content (TFC)

Total phenolics are naturally produced during the growth and development of plants to protect themselves from biotic stresses such as diseases, insects and environmental stresses (Khang *et al.,* 2016). They can delay or inhibit oxidation process of lipids by inhibiting the initiation or propagation of oxidative chain reactions (Khanum *et al.,* 2015). Flavonoids also act as natural antioxidants (Bouaziz *et al.,* 2005). They are wide spread plant secondary metabolites including flavones, flavanols and condensed tannins. Epidemiological studies suggest that the consumption of flavonoid-rich foods protects against human diseases which are associated with oxidative stress. In vitro,

Table 7: Significance of Correlation between protein, amino acid, TPC, TFC and RSA.

	Correlation				
	Protein	Total amino acid	TPC	TFC	RSA
Protein	1				
Total amino acid	0.98*	1			
TPC	0.71*	0.72*	1		
TFC	0.64*	0.64*	0.73*	1	
RSA	0.05	0.11	0.13	-0.13	1

*Cluster analysis done by using NTSYS ver 2.0 software.

Table 8: Cluster analysis of chickpea genotypes based on bio--chemical analysis.

Group	No. of cluster	Name of genotype
Cluster-1	9	JG- 74, JG-14 JGK-2, JG-11, VIJAY, JG-6, RVG-203, GG 5, RSG- 888.
Cluster-2	7	JG-12, JG-315, RVSSG, JAKI-9218, RVG-202, JG-36, BHUPDA CHANA
Cluster-3	14	KRIPA, RVSSG-202, ICC-4812, RVSSG-203, MNK-1, GBM-2, BGD-128, JG-16, RVSSG204, ICCV-2, JG-218, KAK-2, RVKG-101, ANNAGIRI,
Cluster-4	11	JGK-1, RVSSG-205, JG-63, JG-62, JGK-5, JGG-1, RVSSG-24, RVSSG-30, RVG-201, JG-322, DINDORI
Cluster-5	3	DOLLAR, PKV-4, GCP-101

flavonoids from several plant sources have shown free-radical scavenging activity and protection against oxidative stress. TPC ranged highest in Jaki9218 (1.22± 0.8 mg/g) and lowest in Bhupda Chana (0.74 mg/g) (Table 6 and Fig 2). Marathe *et al*. (2011) reported that phenolic content in different legumes lablab bean, chickpea, lentils, cowpea, greengram, pigeonpea and horsegram ranged from 0.325 to 6.378 mg/g. TFC varied from 0.39±1.6 mg/g (ICCV-2) to 0.61± 0.6 mg/g (JAKI-9218) with mean value of 0.47 mg/g.

Scavenging activity

DPPH is a stable free radical with maximum absorbance at 517 nm in methanol. It is used to determine antioxidant activity in natural compounds and its assay is mainly based on an electron transfer reaction and hydrogen-atom abstraction. DPPH free radical scavenging activity in chickpea genotypes ranged from 36.2 to 49.5% with mean value of 40.86%. DPPH scavenging activity in desi and kabuli genotypes indicating their antioxidative potential which might help to reduce oxidative stress in them (Table 6 and Fig 2). Gupta *et al.,* (2017) reported that DPPH radical scavenging activity in forty chickpea genotypes ranged from 32.6 to 58.9%. The protein content (%) significantly correlated with total amino acid, TPC, TFC and RSA. Total amino acid significantly correlated with TPC and TFC and TPC significantly correlated with TFC at 5% significant level (Table 7).

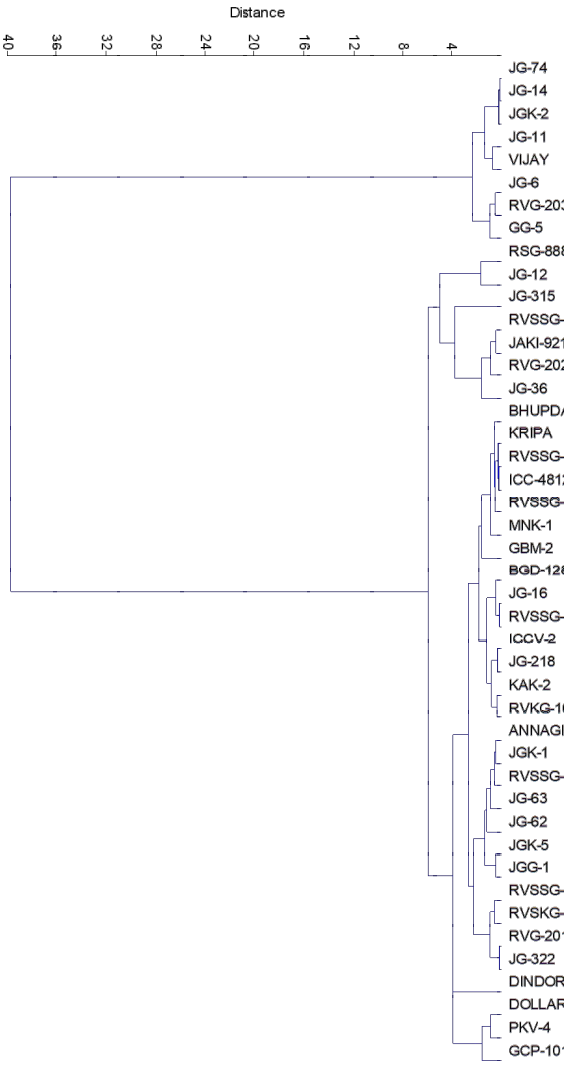

Fig 3: Dendrogram of chickpea genotypes based on biochemical parameters.

Dendrogram based on biochemical parameters

Cluster analysis and grouping of 44 chickpea genotypes was done to observed correlation and similarity between these genotypes based on total protein, total amino acids, TFA, TPC and RSA. Total five clusters has been formed based on these parameters (Fig 3 and Table 8). Cluster I representing all desi chickpea including JG- 74, JG-14 JGK-2, JG-11, VIJAY, JG-6, RVG-203, GG 5, RSG- 888. These varieties are similar in biochemical properties analysed in current study. Other Clusters are making groups of desi and kabuli both.

Characterization of proteins

We have evaluated the crude chickpea protein of 44 genotypes including both desi and kabuli. The seed protein was estimated in each genotypes and it varied from 18 to 26 % respectively (Table 6). The identical amount of protein was loaded on SDS-PAGE using vertical gel electrophoresis

system. Significantly different banding patterns based on molecular weight, were observed among the chickpea genotypes evaluated. In present work, the estimated molecular weights of proteins were 10, 15, 25, 35, 55, 70, 100 and 130 kda and was observed by using ladder 10 to 250 kDa size (Fig 4). Based on protein ladder it was found that 130 kDa bands absence in JG 63 and was presence in all other 43 genotypes. Other than that 100 kDa protein was absence in JG63 and JG12 genotypes, 70 kDa protein was absence in JG63, GG5 and RVSSG-205, RVSSG-

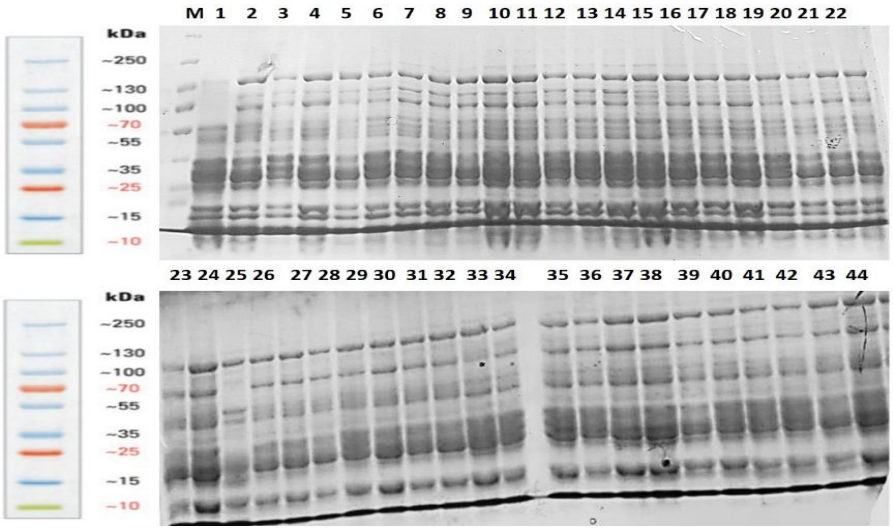

Fig 4: SDS-PAGE profiles (1D) of chickpea genotypes.

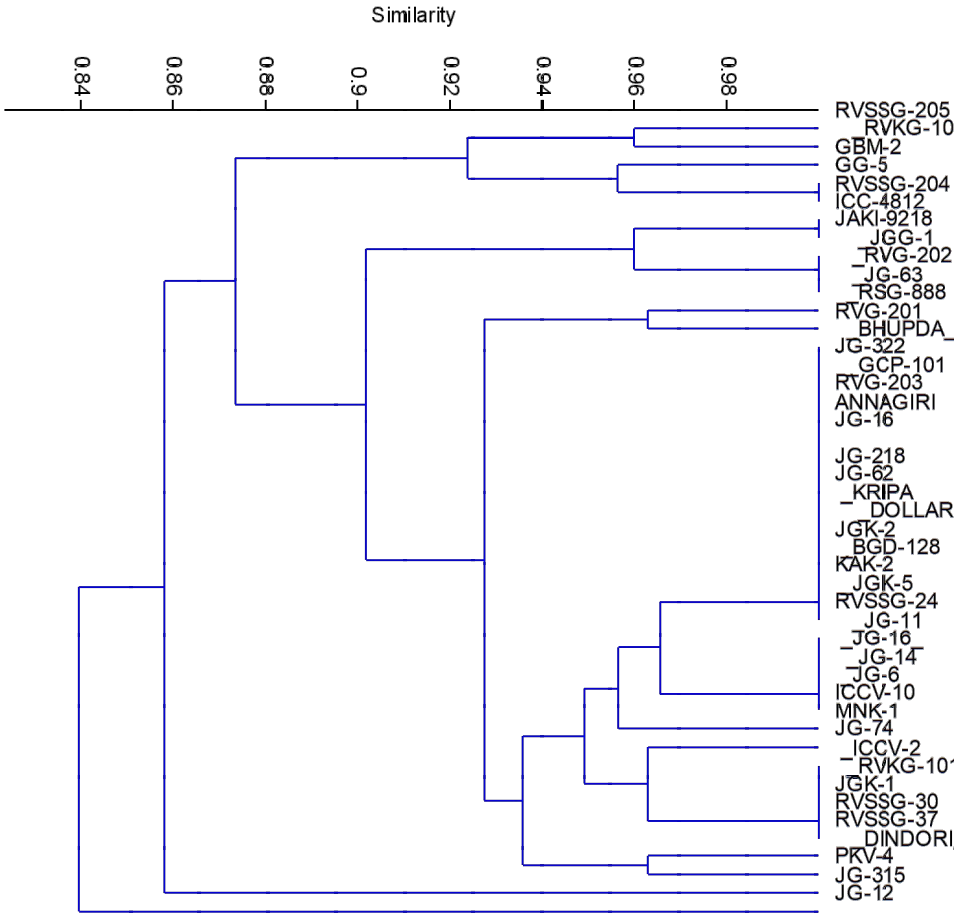

Fig 5: Cluster analysis of chickpea genotypes for protein.

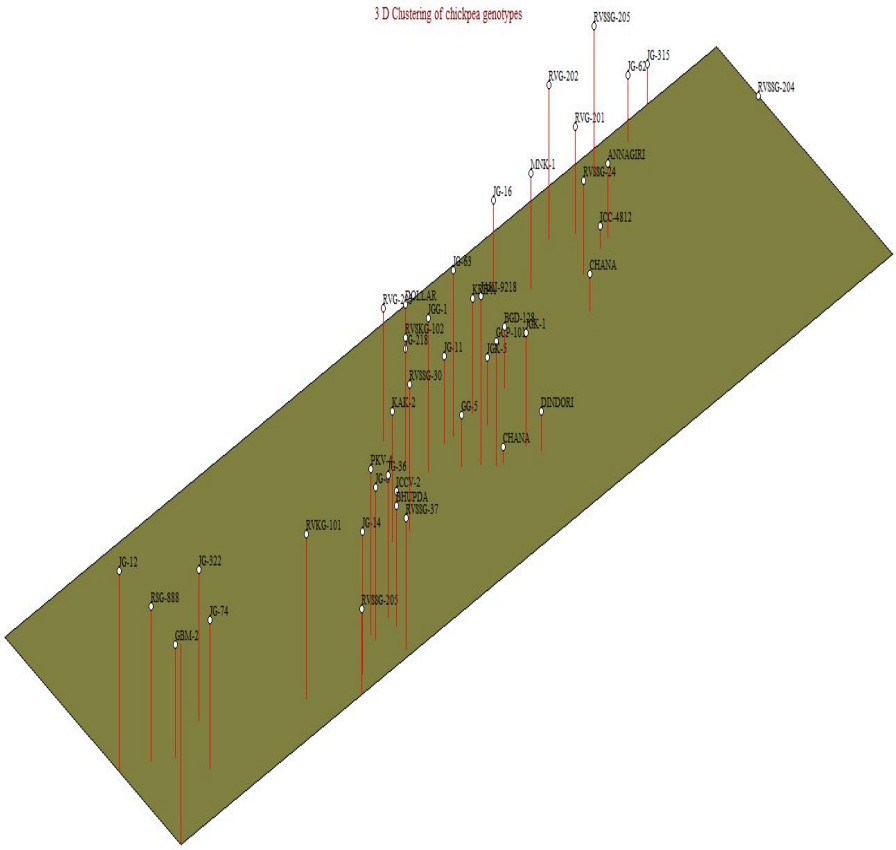

Fig 6: Clustering (3D) of chickpea genotypes for leaf area index, chlorophyll content, Total Phenolic Contents, Total Flavonoid Contents, Radical scavenging activity and protein content.

Table 9: Significance of Correlation between protein, amino acid, TPC, TFC, RSA, leaf area and total chlorophyll content in chickpea genotypes.

Correlations								
	Protein	TAA	TPC	TFC	RSA	LA_30	LA_60	TC
Protein								
TAA	0.979**							
TPC	0.711**	0.725**						
TFC	0.640**	0.642**	0.729**					
RSA	0.449**	0.422**	0.212	0.388**				
LA_30	0.259	0.207	-0.111	-0.153	0.092			
LA_60	-0.157	-.144	-0.249	-0.112	0.424**	-0.128		
TC	0.014	0.039	-0.082	-0.102	-0.157	0.170	-0.338*	

**. Correlation is significant at the 0.01 level (2-tailed).*. Correlation is significant at the 0.05 level (2-tailed).

* Protein, Total Amino acid (TAA), Total Phenolic Contents (TPC), Total flavonoid content (TFC) and radical-scavenging activity (RSA) represented in %, mg/g, mg/g, mg/g and % respectively.

204, GBM-2, JG62, KRIPA and DOLLAR. Specific protein of 55 kDa was found in all 43 chickpea genotypes, but in Bhupda chana faint band was observed. Protein present between 35 to 25 kDa were showing multiple bands but some genotypes ICC-4812, JAKI-9218, JG-315, GG-5, RVSSG-205, RVSSG-204, GBM-2 were giving faint bands. One bands absent in JG12 chickpea. Proteins of 10 kDa was mostly present in all chickpea genotypes. Desi genotypes were mostly presenting diverse size of protein but kabuli chickpea bands were mostly similar in size. Cluster analysis of protein pattern represented 7 groups, representing similar protein pattern in these chickpea varieties (Fig 5). Similar to biochemical analysis Cluster I represented mostly desi chickpea including RVSSG-205, RVKG-10, GBM-2, GG-5, RVSSG-204, ICC-4812, so these genotypes may have similar proteins. Pooled analysis of our result indicated that

leaf area index at 60 days is highly positively significantly correlated with RSA (r=0.424), RSA with protein and TFC and TFC with protein and TPC (Table 9) at 1%significance level. Pooled analysis of all the observed biochemical and physiological observation formed 5 groups of chickpea genotypes (Fig 6). Desi chickpea JG12 is most distinct from the variety RVSKVV 204 showing their diverse characters in respect to all the analyzed parameters.

The seeds of desi chickpeas are usually small and dark colored with reticulated surface and the aerial plant parts usually anthocyanin pigmented bearing pink or purple flowers. The desi type is considered to be primitive compared to the recent origin of kabulis. Both these types had been geographically isolated for many years (Gowda *et al*., 1987). Despite vast morphological differences between the desi and kabuli genotypes, it has found that these types are lot more common. For example, out of 1683 alleles detected in the composite collection of chickpea, 436 were common between the two types and the frequency of common alleles between desi and kabuli types were shown to range from 47% to 54% (Upadhyaya *et al*., 2008). Kabuli and desi germplasm were shown to have similar mean gene diversity, while the kabuli's as a group were genetically more diverse than desi. Desi were shown to contain the largest number of unique alleles and a higher proportion of rare alleles compared to Kabulis. Purushothaman *et al* 2014, shown that kabuli seeds are more nutritive in respect to their protein content however, chickpeas with dark seed coat (desi) are known for their higher antioxidant activity, arising from the phenolics fraction (Segev, 2011 *et al*.). The objective of the current study was to compare the total protein, total amino acid, total phenolic content (TPC), total flavonoid content (TFC) and free radical scavenging activity content in a set of 29 desi and 15 kabuli chickpea genotypes. We found more protein, TPC, TFC and RSA in Desi chickpea varieties JG315, Jaki9218, Jaki9218 and JG12 respectively. Macar *et al*. (2017) screened grains of a desi (ICC 3996) type and three kabuli types spectrophotometrically for their total phenolic, flavonoid and protein contents and reported that total phenolic level of chickpea grains may be associated with seed colour. In our study we found more TPC, TFC and RSA in desi chickpea, supported the results of Macar *et al* (2017). Although Segev *et al*. (2011) reported that seed coat usually contains more than 95% of phenolics and flavonoids, our desi chickpea varieties showed more phenolics and flavonoid content as compared to kabuli varieties.

CONCLUSION

The result of our study indicated diverse biochemical values in desi and kabuli chickpea for observed traits. Total protein varied from 18.2 (Dollar variety) to 26.7 % (JG315), total Amino acid from 3.8 (Dollar variety) to 6.8 mg/g (JG315), total TPC from 0.74 (Bhupda Chana) to 1.22 mg/g (Jaki9218), total TFC content from 0.39 (ICCV2) to 0.61 mg/g (Jaki9218) and total RSA ranged from 36 (Dhindori) to 47% (JG12). Highest protein and amino acid was observed in JG315, while Jaki 9218 presented highest total phenolic (TPC) and flavonoid (TFC) content. Desi variety JG12 indicated highest percentage of Radical scavenging activity. No Significant correlation was found between leaf area index and total chlorophyll content although Chl b is negatively significantly correlated with LAI at 1% significant level. Pooled analysis of all the biochemical and physiological parameters indicated that leaf area index at 60 days is highly positively significantly correlated with RSA (r=0.424), RSA with protein and TFC and TFC with protein and TPC at 1% significance level. Identified genotypes with higher protein, amino acid, TPC and TFC would be used for further varietal improvement programme of chickpea. Analysed chickpea genotypes of desi and kabuli can be considered as therapeutic functional foods due to phenolics, flavonoids, RSA and rich protein content. However further more studies are required to characterize other antioxidant properties of chickpea grains.

Conflicts of interest

The authors declare no conflicts of interest.

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