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Comparative Analysis of Photosynthetic and Biochemical Characteristics of Desi and Kabuli Genepools of Chickpea *(Cicer arietinum L.)*

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Abstract

Chickpea is one of the most heat and drought tolerant crop among the cool-season legumes and grown under diverse geographic conditions. Since both gene pools of chickpea viz desi and kabuli differ substantially from each other and not much work has been done in photosynthetic parameters and many other aspects of each varieties, therefore a comparative study was done to gain fundamental insights of photosynthetic parameters, stress responsive factors and antioxidants in desi and kabuli genepools in chickpea. Desi varieties accumulated significantly higher levels of chlorophyll, carotenoids and had reduced photosynthetic efficiency (Fv/Fm), electron transport rate (ETR), quantum yield (Y), activity of photosystem-I as compared to kabuli varieties grown under similar condition. Soluble proline accumulation and lipid peroxidation in terms of MDA production was significantly high in desi varieties. Ascorbate peroxidase activity was lower whereas superoxide dismutase, glutathione peroxidase activities of desi seedlings were significantly higher than kabuli. Salt stress of 200mM NaCl was tolerated more efficiently by desi seedlings as compared to kabuli seedlings. These results demonstrate that desi gene pool as compared to kabuli gene pool had reduced photosynthetic parameters and high oxidative stress and proline accumulation. The high amount of proline and carotenoids accumulated by desi seedlings enhanced its ability to tolerate salt stress more efficiently than kabuli seedlings. This is a preliminary data which shows that these variations might have occurred because desi gene pool has a wide range of adaptation and widespread cultivation in semi-arid and tropical regions.

Keywords: Chlorophyll fluorescence; photosynthesis; photosynthetic efficiency; electron transport.

Introduction

Chickpea (*Cicer arietinum L.)* is the third most important grain legume crop and a rich source of plant based dietary protein. Seed size and colour varies has led to the designation of the two primary types: Kabuli and Desi. Desi chickpea varieties were attributed with pink flowers, small, angular, dark, colored seeds while the Kabuli varieties have white flowers, large, smooth coated, beige seeds cream to white in color. It is commonly accepted that Kabuli chickpeas originated from the desi types in the mediterranean basin and represent macrosperma and microsperma genepools within chickpea (Singh and Saxena 1996). These two races also differ in many characters of agronomic value and differ in many physico-chemical seed characters. The average world production of chickpea is 0.71t ha-1 which is far below the yield potential. It is generally observed that countries producing kabuli chickpeas have higher yield averages than the desi varieties (Singh and Saxena 1999). Previous studies have shown that during the early stages of seed development pod walls of chickpea can fix CO2 under well-irradiated conditions, but generate large losses of CO_2 through respiration in the dark (Sheoran et al. 1987) although photosynthesis by the external pod wall has been suggested to contribute to seed growth in chickpea (Sheoran et al. 1987).

Plants sense many aspects of light in their environment including its wavelength, duration, intensity, and direction for their growth and development. Despite the necessity of light for autotrophic organisms, no plant is capable of using 100% of the solar irradiation for photosynthesis (Demming-Adams et al. 1998). Most plants have the ability to acclimate to a specific light environment during which chlorophyll concentration, calvin cycle intermediates, chloroplast density, and carotenoid composition alters (Demming-Adams et al. 1998). The utilization and dissipation of light energy by plant is function of both genotypic and environmental interactions. Therefore a larger percentage of absorbed energy is used in photosynthesis, reducing the need for alternative dissipating mechanism, and minimizing the risk of photo-damage (Kitao et al. 2000).

A few parameters which can affect the productivity of higher plants are determined by the incident photosynthetic photon flux (PPF) and the efficiency of the following four physiological processes: (1) absorption of PPF by photosynthetic tissue, (2) carbon fixation (photosynthesis), (3) carbon use (respiration), and (4) carbon partitioning (harvest index). The constituent processes are analysed to determine theoretic, potential and achievable productivity. Crop productivity i.e., grain yield and seed size (kg/hc) of desi is lesser than kabuli. Bioproductivity may be dependent upon the transformation of solar energy to chemical energy and on improvement in quality of plants and their resistance to unfavourable environmental factors (Mostowska 1997). A comparative study was done between desi and kabuli gene pools of chick pea (*Cicer arietinum L.*) to analyze a few parameters which can give some insights for the differences in the two gene pools.

Materials and Methods

Plant material and growth condition:

Chickpea (Cicer arietinum L.) desi and kabuli varieties seeds were used as experimental materials. Accessions of desi variety used were JG62, ICC16915 and ICC14098 and kabuli were ICCV2, ICC13816 and ICC15264 of chickpea (Cicer arietinum L.). These varieties were obtained from ICRISAT, Hyderabad from their seed bank where their germplasm was stored and were pure lines of desi and kabuli genepools of chickpea (*C. arietinum L.*). Seeds were grown in pots in natural environment in half strength Hoagland solution treated mixture of agropeat and vermiculite with 12hL/12hD photoperiod with maximum temperature of 22-28^{0}C and minimum temperature of 16-20^{0}C. 21days old plants were used for each experiment and analysis was done in triplicates.

Chlorophyll, Carotenoid and Protein Estimation

Chl and carotenoid contents were estimated as described previously (Wellburn and Lichtenthaler 1984, Porra et al. 1989). Young leaves were taken in the morning from the same branch and estimations of 3 varieties each of desi and kabuli was done. Each experiment was done in triplicates and results were analysed by students t-test at 95% confidence level using SPSS software . Total protein content was estimated according to Bradford (1976).

Spectrofluorimetric analysis

Chlorophyll fluorescence was measured on dark adapted leaves using a pulse modulated fluorometer PAM 2100 (Hansatech Instruments, H. Walz, Effeltrich, Germany) from three weeks old leaves of similar side and position.

Soluble proline estimation

Free proline was determined according to the method of Bates et al. (1973). 5 g of tissue was homogenized in 10 ml of 3% salicylic acid, and centrifuged at 4000g for 10 min. 2 ml of acid ninhydrin was added into 2 ml of supernatant, followed by the addition of 2 ml of glacial acetic acid and boiled for 60 min. The mixture was extracted with toluene, and free proline was quantified spectrophotometrically at 520 nm from the organic phase.

Fatty acid peroxidation measurement

Malondialdehyde (MDA), as end product of lipid peroxidation, was measured using the thiobarbituric acid (TBA) test. 200 mg of fresh tissue was extracted in 0.25% thiobarbituric acid in 10% TCA. Contents were heated at 95^{0}C for 30 min, then the reaction was stopped in an ice bath, than centrifuged at 10,000 rpm for 10 min. Absorbance of supernatant was measured at 532 nm and correction for nonspecific turbidity was done by substracting the absorbance of the same at 600 nm. The amount of MDA was calculated using an extinction coefficient of 155 mm^{-1} cm^{-1}. The concentration of lipid peroxides along with oxidatively modified proteins was measured according to Heath and Packer (1968).

Assay of antioxidant enzyme activities

Superoxide dismutase (SOD, EC. 1.15.1.1) activity was determined by spectrophotometric method based on the inhibition of O_2-driven NADH oxidation at 340 nm at 25^0 C using an extinction coefficient of 6.2 $mM^{-1}cm^{-1}$ (Paoletti et al. 1986). Ascorbate peroxidase (APX, EC 1.11.1.11) was assayed from the decrease in absorbance at 290 nm using an extinction coefficient of 2.8 mM-1cm^{-1} as ascorbate is oxidized (Asada and Nakano 1988). The activity of gluthatione reductase (GR, EC 1.6.4.2) was determined based on its catalyzing effect on NADPH-dependent reduction of glutathione disulfide (GSSG) to glutathione (GSH) at 340 nm using an extinction coefficient of 6.2 $mM^{-1}cm^{-1}$ (Carlberg and Mannervik 1985). The estimations were done in triplicates for each variety of desi and kabuli. The values are expressed are mean of the estimations done for desi and kabuli varieties. The results were analysed using students t-test at 95% confidence level using SPSS software.

Salt treatment of chickpea seedlings

Chickpea seeds were germinated and grown under normal light condition. 10 days after grown in light they were transferred to 100 and 200 mM salt containing hydroponic cultures. The photosynthetic efficiency (Fv/Fmax) were measured after 48 hours of growth in NaCl solutions. Both 10 days old desi and kabuli genepools poorly tolerated salt strength above 200mM.

Results

Chlorophyll (Chl) and carotenoid accumulation.

Total Chl content was significantly different in different accessions of desi and kabuli chickpea varieties. Seedlings of desi varieties JG62, ICC16915 and ICC14098 when grown in light accumulated higher amounts of Chl and carotenoid as compared to kabuli varities ICCV2, ICC13816 and ICC15264 of chickpea *(Cicer arietinum L.).* Chl accumulation of desi varieties was significantly higher than kabuli varieties (Fig. 1A). Similar trend was observed in carotenoid accumulation also (Fig. 1A).

Light energy drives primary photochemical reactions that initiates the photosynthetic energy conversions**.** Fluorometric parameters of different desi and kabuli varieties were analysed and it revealed that ratio of variable fluorescence to maximum fluorescence (Fv/Fm) which shows the intactness and efficiency of PS-II was lower in desi varieties as compared to kabuli varieties under similar growth conditions (Fig.1B). In these three weeks old desi plants other parameters like rate of electron transport (ETR), quantum yield (Y) were low when compared to the three kabuli varieties (Fig.2A and Fig. 2B).

Photochemical quenching (qP) of desi seedlings was low at high PAR (except ICCV2; Fig.2C) and non photochemical quenching parameter (NPQ) was higher in desi seedlings (Fig.2D). PSI activity when estimated from crude chloroplast extract showed significant reduction ($p<0.05$) in all the three desi varieties of chickpea (Fig. 3).

Lipid peroxidation and level of soluble proline

MDA (one of the major TBA reactive metabolite) production when compared in desi and kabuli varieties showed significantly high ($p<0.05$) accumulation in desi varieties (Fig. 4A). Soluble proline estimation showed that desi varieties (JG62, ICC16915 & ICC14098) of chickpea accumulated substantially and significantly higher amount ($p<0.05$) of soluble proline as compared to kabuli (ICCV2, ICC13816 & ICC15264) varieties (Fig. 4B).

Scavengers of Free Radicals:

SOD activity, responsible for the elimination of superoxide radicals in cells, increased significantly in desi chickpea ($p<0.05$) (Fig. 5A). The enzyme activity of APX - which hydrolyses H_2O_2 - in desi variety was significantly lower than kabuli variety ($p<0.05$) (Fig. 5B). GR, that catalyzes the NADPH-dependent reduction of oxidized glutathione, showed significant increase ($p<0.05$) in its activity in desi chickpea (Fig. 5C).

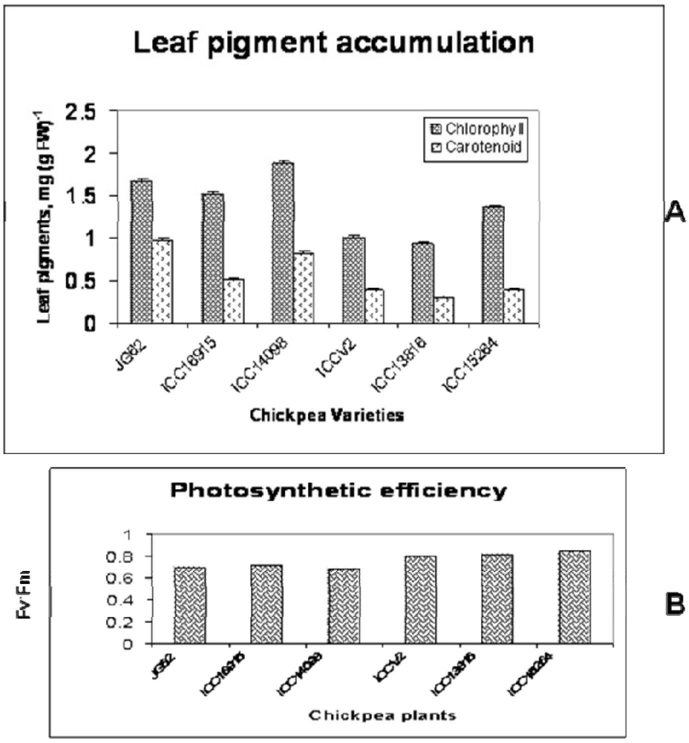

Figure 1. A) Shows chlorophyll and carotenoid accumulation and B) Shows Photosynthetic efficiency (Fv/Fm) of 21d old chickpea (*Cicer arietinum. L*) seedlings. Seedlings of JG62, ICC16915, ICC14098 of desi and ICCV2, ICC13816, ICC15264 of kabuli variety of chickpea were used. Leaves of same branch and same growth stage were used for measurements, SEM is represented as error bar. The estimations were done in triplicates and mean values of each parameter was used as final reading. All the measurements were performed as described in “Materials and methods”.

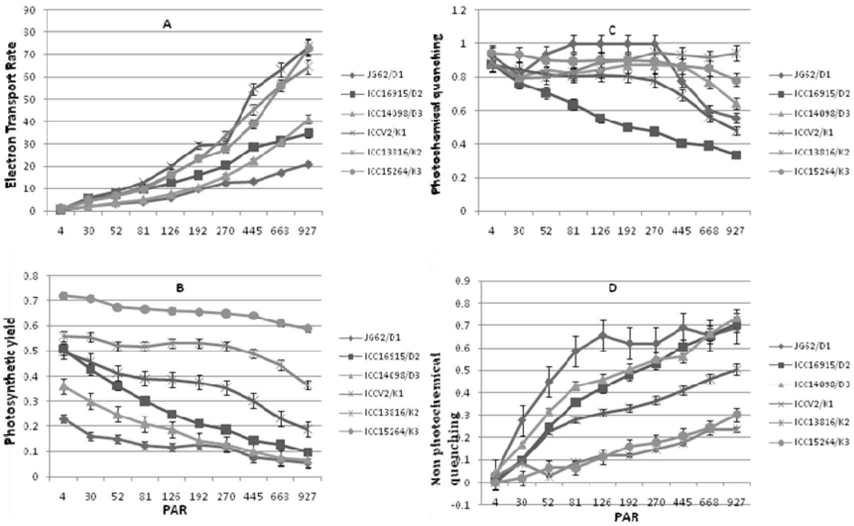

Figure 2. A) Electron transport rate (ETR), B) photosynthetic yield, C) photochemical quenching, D) Non-photochemical quenching at different photosynthetically active radiation (PAR) from 21-d-old desi and kabuli chickpea (*Cicer arietinum. L*) seedlings were measured. Seedlings of JG62, ICC16915, ICC14098 of desi and ICCV2, ICC13816, ICC15264 of kabuli variety of chickpea were used. Leaves of same branch and same growth stage were used for measurements, SEM is represented as error bar. All the measurements were performed as described in "Materials and methods".

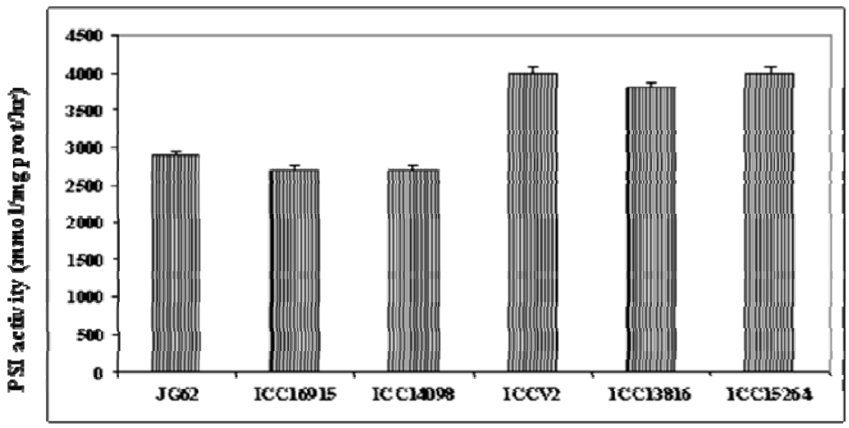

Figure 3. Shows the activity of photo system- I in 21-d-old seedlings from desi and kabuli chickpea *(Cicer arietinum. L).* Seedlings of JG62, ICC16915, ICC14098 of desi and ICCV2, ICC13816, ICC15264 of kabuli variety of chickpea were used. Leaves of same branch and same growth stage were used for measurements. The estimations were done in triplicates and mean values of each parameter was used as final reading, SEM is represented as error bar. All the measurements were performed as described in "Materials and methods".

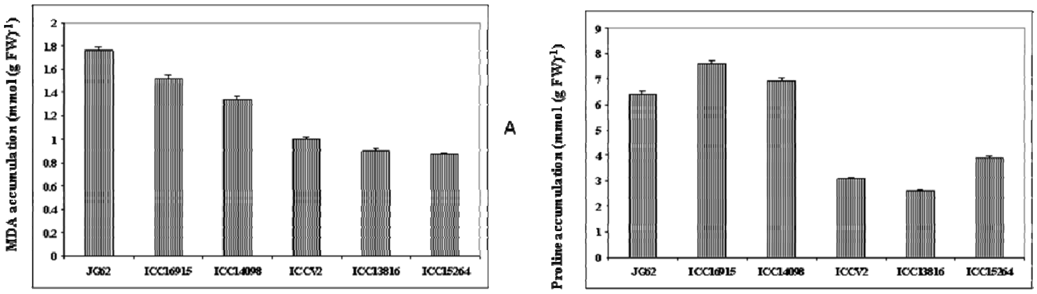

Figure 4. A) Malondialdehyde (MDA) accumulation and B) Soluble proline content of 21-d-old desi and kabuli chickpea (*Cicer arietinum. L*) seedlings were measured. Seedlings of JG62, ICC16915, ICC14098 of desi and ICCV2, ICC13816, ICC15264 of kabuli variety of chickpea were used. The estimations were done in triplicates and mean values of each parameter was used as final reading, SEM is represented as error bar. All the measurements were performed as described in "Materials and methods".

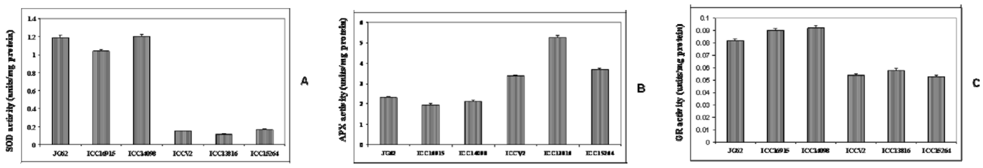

Figure 5. The enzyme activities of A)Superoxide dismutase (SOD), Ascorbate peroxidise (APX), and Glutathione reductase (GR) was measured from the soluble protein extract of 21-d-old leaves of JG62, ICC16915, ICC14098 of desi and ICCV2, ICC13816, ICC15264 of kabuli variety of chickpea (*Cicer arietinum L.*). The estimations were done in triplicates and mean values of each parameter was used as final reading, SEM is represented as error bar. All the measurements were performed as described in "Materials and methods".

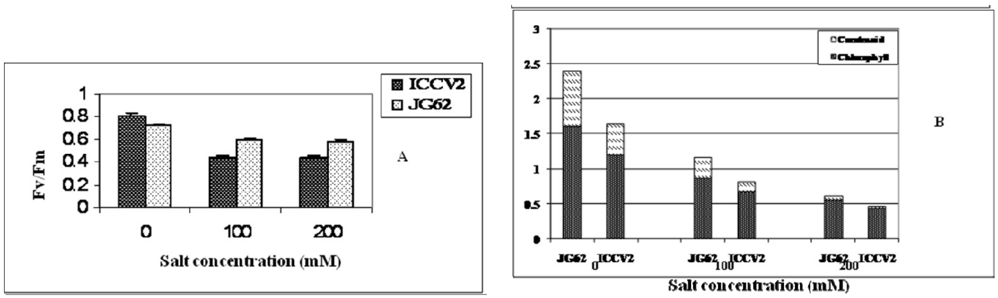

Figure 6. A) The figure shows the values for Fv/Fm and B) chlorophyll and carotenoid content in 10-d-old chickpea seedlings grown for 48 h in 0, 100 and 200mM NaCl concentration hydroponically. The 10-d-old seedlings of JG62 of desi and ICCV2 of kabuli variety of chickpea (*Cicer arietinum L.*) were used. The estimations were done in triplicates and mean values of each parameter was used as final reading. All the measurements were performed as described in "Materials and methods".

Effect of salt concentrations

Under optimal condition cellular homeostasis is maintained at normal levels by the combined action of various metabolic pathways. Since desi chickpea is normally (without any external stress) accumulating significantly higher amount of soluble proline as compared to kabuli genotype, so we were interested in checking whether this factor makes it more tolerant to osmotic stress. The stress tolerance capacities of both chickpea varieties were measured by treatment with different concentrations of sodium chloride for 48 hours. Photosynthetic efficiency in terms of Fv/Fm after salt treatment was less affected in desi varieties as compared to the kabuli varieties (Fig. 5A). The chl and carotenoid accumulation was also reduced after 100 and 200mM NaCl treatment, but was more in desi seedlings as compared to kabuli seedlings (Fig. 5B).

Discussion

Chickpea (Cicer arietinum L.) seedlings of desi and kabuli varieties accumulated substantial Chl and carotenoid. However, the Chl as well as carotenoid content of desi varieties was significantly higher as compared to kabuli varieties. Photons are absorbed by molecules of antenna pigments, the excitation energy is transferred to reaction centers of the photosystems. The energy drives primary photochemical reactions that initiates the photosynthetic energy conversion in photochemical and biochemical pathway. So an increase in the percentage of absorbed photons should be responsible for an increased yield (Bugbee and Salisbury 1989). Carotenoids are now recognized as having key roles in both light harvesting and photoprotection (Siefermann 1985; Siefermann 1987; Frank et al. 1991; Frank and Christensen 1995; Koyama 1991). Of particular importance is the role of carotenoids in protecting the photosynthetic apparatus from photodamage either by nonphotochemical quenching of Chl fluorescence in the light harvesting complexes or by quenching of O2 in the reaction center.

Chlorophyll increase is also reported under osmotic stress (saline stress=osmotic stress) (Valenzuelaa et al. 2005). Chlorophyll fluorescence is used to determine the state of energy distribution in the thylakoid membrane, the quantum efficiency of PS-II, and the extent of photoinhibition (Bjorkman and Demmig-Adams 1994; Critchley 1998; Maxwell and Johnson 2000). In desi variety of chickpea quantum efficiency of PS-II (Fv/Fm), photosynthetic electron transport rate (ETR), quantum yield (Q), and photochemical quenching coefficient was affected as compared to kabuli variety. This might result in reduced efficiency of absorbed light energy transfer from the light harvesting complex to PS-II (Bilger and Bjorkman 1990; Maxwell and Johnson 2000; Ranney and Peet 1994). Plants subjected to high irradiance stress typically have lower Fv/Fm values than non-stressed plants. Higher non photochemical quenching coefficient in desi chickpea is suggestive of its more adaptive capacity to a higher irradiance which may be due to its evolution from winter crop to summer crop. Qp and NPQ coefficients can provide a quantitative expression of the susceptibility of PS-II to photoinhibition and decline in PS-II activity (Osmond 1994; Park et al. 1995; Park et al. 1996; Ogren 1991). The activities of PS-II as revealed by chlorophyll fluorescence was lower in desi varieties. The PS-I activity was significantly lower in desi seedlings when compared to kabuli seedlings. The quantitative relationship between chlorophyll fluorescence and the efficiency of photosynthetic energy conversion opens new ways of application in plant breeding and evaluation of plant productivity (Schreiber et al. 1995; Baker et al. 2001). Our results are preliminary and they suggest that the low photosynthetic efficiency, PS-I activity, ETR, and photosynthetic yield might affect the yield of desi plants.

Under high irradiance, the transfer of absorbed energy becomes particularly important because excess energy can lead to the formation of reactive oxygen species (Critchley 1998; Schansker and Van Rensen 1999). MDA accumulation is significantly high in desi seedlings. MDA is a cytotoxic peroxidation product of lipids, and is the likely agent responsible for membrane disruption suggesting that considerable reactive oxygen species are being produced in desi seedlings. Activated forms of O_2 are implicated in the O_2-dependent damage to the membrane. SOD level being higher in desi seedlings is suggestive of the protection in the presence of the O_2 radical. O^-_2 can be produced from the photosynthetic ETC due to univalent reduction of O_2 by the reductants generated at the acceptor site of PS-I. So the O2-produced is disproportionated by SOD and H_2O_2 thus produced is reduced to H_2O by one molecule of ascorbate mediated by ascorbate peroxidase. The dehydro ascorbate (DHA) produced in the above reaction is reduced to ascorbate by the reductant glutathione and oxidized glutathione thus produced is regenerated by NADPH-dependent glutathione reductase. Substantial increase in the activity of SOD and small increase in the activity of GR in desi seedlings suggest their involvement and imparting protection to desi seedlings against photooxidative stress. Reduction in the activity of ascorbate specific peroxidase in desi seedlings suggest that it is affected in desi chickpea seedlings. Increase in peroxides could be due to reduction of ASP, or due to breakdown of hydroperoxides generated by O_2^-.

Soluble proline which acts as osmolyte and protects plants against stress is high in desi seedlings. Proline may physically quench singlet oxygen or reacts directly with the hydroxyl radicals (Rustgi et al. 1977; Floyd and Nagy 1984; Lissi et al. 1993; Alia et al. 2001). A large body of data indicate a positive correlation between proline accumulation and adaptation to stress leading to increased osmotolerance (Csonka 1989, Szekely et al. 2008). Desi seedlings produce high amounts of soluble proline and carotenoids. They had been implicated in quenching excessive light as well as scavenging reactive oxygen species thus protecting plants. Desi seedlings were accumulating more proline and they tolerated salt stress of 200 mM more efficiently than kabuli seedlings after 48h of salt treatment.

In conclusion desi and kabuli gene pools differed substantially in various parameters, these differences might be because of adaptation of desi gene pools to semi-arid and tropical regions.

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References

[1] Alia, Mohanty P, Matysik J (2001) Effect of proline on the production of singlet oxygen. Amino Acids 21: 195-200.

[2] Asada K, Nakano M (1988) Production of active oxygen by autooxidation. Tanpakushitsu Kakusan Koso 33: 2776-80.

[3] Baker NR, Oxborough K, Lawson T, Morison JIL (2001) High resolution imaging of photosynthetic activities of tissues, cells and chloroplasts in leaves. J Exp Bot. 52: 615-621.

[4] Bates LS, Waldren RP, Teare LD (1973) Rapid determination of free proline for water stress studies. Plant Soil 9: 205-207.

[5] Bilger W, Bjorkman O (1990) Role of the xanthophylls cycle in photoprotection elucidated by measurements of light-induced absorbance changes, fluorescence and photosynthesis in leaves of Hedera canariensis. Photosyn Research 25: 173-185.

[6] Bjorkman O, Demmig-Adams B (1994) Regulation of photosynthetic light energy capture, conversion, and dissipation in leaves of higher plants, In ED Schulze, MM Caldwell (eds) Ecophysiology of Photosynthesis. Ecological Studies 100, Springer, Berlin, pp 17-47.

[7] Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 72: 248-254.

[8] Bugbee BG, Salisbury FB (1989) Current and potential productivity of wheat for a controlled environment life support system. Adv Space Res 9: 5-15.

[9] Carlberg I, Mannervik B (1985) Glutathione reductase. In A. Meister (ed) Methods in enzymology vol 113, Academic, Orlando, pp. 484–90.

[10] Critchley C (1998) Photoinhibition, In: A.S. Raghvendra (ed) Photosynthesis: A comprehensive treatise. Cambridge, United Kingdom, pp264-272.

[11] Csonka LN (1989) Physiological and genetic responses of bacteria to osmotic stress. Microbiol Review 53: 121-147.

[12] Demmig-Adams B, Moeller DA, Logan BA, Adams WW III (1998) Positive correlation between levels of retained zeaxanthin + antherazanthin and degree of photoinhibition in shade leaves of *Schefflera arboricola (Hayata) Merrill*. Planta 205: 367-374.

[13] Floyd RA, Nagy I (1984) Formation of long lived hydroxyl-free radical adducts of proline and hydroxyproline in a fenton reaction. Biochim Biophys Acta 790: 94-97.

[14] Frank HA, Christensen RL (1995) Singlet energy transfer from carotenoids to bacteriochlorophylls, In R.E. Blankenship, M.T. Madigan and C.E. Bauer (eds) Anoxygenic photosynthetic bacteria, Kluver, Netherlands, 373-384.

[15] Frank H, Violette CA, Trautman JK, Shreve AP, Owens TG, Albrecht AC (1991) Photosynthetic carotenoids: structure and photochemistry. Pure Appl Chem 63: 109-114.

[16] Heath RL, Parker L (1968) Photoperoxidation in isolated chloroplasts. I. Kinetics and stoichiometry of fatty acid peroxidation. Arch Biochem Biophys. 125: 189-98.

[17] Kitao M, Lei Koike TT, Tobita H, Maruyama Y (2000) Susceptibility to photoinhibition of three deciduous broadleaf tree species with different successional traits raised under various light regimes. Plant Cell Environ 23: 81-89.

[18] Koyama Y (1991) Structures and functions of carotenoids in photosynthetic systems. J Photochem Photobiol. 9: 265-280.

[19] Lissi EA, Encinas MV, Lemp E, Rubio MA (1993) Singlet O_2 bimolecular processes: solvent and compartmentalization effects. Chemical Reviews 93: 699-723.

[20] Maxwell K, Johnson GN (2000) Chlorophyll fluorescence - a practical guide. J Exp Botany 51: 659-668.

[21] Mostowska A, (1997) Environmental factors affecting chloroplasts In: M Pesserauli (ed) Handbook of Photosynthesis. New York: Marcel Dekker, pp. 407-426. Muehlbauer FJ, Singh KB (1987) Genetics of chickpea. In: M.C. Saxena and K.B. Singh (eds) The Chickpea. CAB. International, Wallingford, Oxon, OX10 8DE, UK. pp. 99-125.

[22] Ogren E (1991) Prediction of photoinhibition of photosynthesis from measurements of fluorescence quenching components. Planta 184: 538-544.

[23] Osmond CB (1994) What is photoinhibition: some insights from comparisons of sun and shade plants. In Baker NR and Bowler JR (eds) Photoinhibition: Molecular mechanisms to the field, Bios Scientific, UK, pp1-24.

[24] Paoletti F, Aldinucci D, Mocali A, Caparrini A (1986) A sensitive spectrophotometric method for the determination of superoxide dismutase activity in tissue extracts. Anal Biochem 154: 536

[25] Park YI, Chow WS, Andersson JM (1995) The quantum yield of photoinactivation of photosystem II in pea leaves is greater at low than high photon exposure. Plant Cell Physiol 36: 1163-1167.

[26] Park YI, Chow WS, Andersson JM, Hurry VM (1996) Differential susceptibility of photosystem II to light stress in light acclimated pea leaves depends on the capacity for photochemical and non-radiative dissipation of light. Plant Science 115: 137-149.

[27] Porra RJ, Thompson WA, Kriedemann PE (1989) Determination of accurate extinction coefficients and simultaneous equations for assaying chlorophylls a and b extracted with four different solvents: verification of the concentration of chlorophyll standards by atomic absorption spectroscopy. Biochim Biophys Acta 975: 384-394.

[28] Ranney TG, Peet MM (1994) Heat tolerance of five taxa of birch (*Betula)*: Physiological responses to supraoptimal leaf temperatures. J American Soc Horticultural Sci 119: 243-248.

[29] Rustgi S, Joshi A, Moss H, Riesz P (1977) ESR of spin trapped radicals in aqueous solutions of aminoacids: reactions of the hydroxyl radical. Internat J Rad Biol Rel Stu Phys Chem Med 31: 415-440.

[30] Schansker G, Van Rensen JJS (1999) Performance of active photosystem II centers in photoinhibited pea leaves. Photosyn Res 62: 175-184.

[31] Schreiber U, Bilger W. Neubauer C (1995) Chlorophyll fluorescence as a nonintrusive indicator for rapid assessment of in vivo photosynthesis. In Schulze, E.D. & Caldwell, M.M. (eds): Ecophysiology of Photosynthesis. Springer-Verlag, Berlin, Heibelberg, pp.49-70.

[32] Sheoran IS, Singal HR, Singh R. (1987) Photosynthetic characteristics of chickpea (*Cicer arietinum L.)* pod wall during seed development. Ind J Exp Biol 25: 843–847.

[33] Siefermann HD (1985) Carotenoids in photosynthesis. I. Location in photosynthetic membranes and light harvesting function. Biochim Biophys Acta 325-355.

[34] Siefermann HD (1987) The light-harvesting and protective functions of carotenoids in photosynthetic membranes. Physiologia Plantarum 69: 561-568.

[35] Singh KB, Saxena MC (1996) Winter chickpea in mediterranean type environments. A technical bulletin. In dry areas Aleppo Syria.

[36] Singh KB, Saxena MC (1999) Geographical distribution and production trends. In Rene C (ed) The Tropical Agriculturalist. Macmillan pp. 1-7.

[37] Székely G, Abrahám E, Cséplo A, Rigó G, Zsigmond L, Csiszár J, Ayaydin F, Strizhov N, Jásik J, Schmelzer E, Koncz C, Szabados L (2008) Duplicated P5CS genes of Arabidopsis play distinct roles in stress regulation and developmental control of proline biosynthesis. Plant J 53:11-28.

[38] Valenzuelaa XG, Moyab EG, Cruza QR, Estrellac LH, Santacruza GAA (2005) Chlorophyll accumulation is enhanced by osmotic stress in graminaceous chlorophyllic cells. J of Plant Physiol 162:650-661.

[39] Welburn AR, Lichtenthaler H (1984) Formulae and program to determine total carotenoids and Chl a and b of leaf extracts in different solvents. In Sybesma C (ed) Advances in photosynthesis research, Martinus N, II, Dr. W. Junk, The Hague/Boston/Lancaster, pp 9-12.