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Impact of osmolytes in pollen viability and yield attributes of chickpea (*Cicer arietinum* L.) under drought and heat environment

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Abstract
The present investigation entitled "Impact of osmolytes in pollen viability and yield attributes of chickpea (*Cicer arietinum* L.) under drought and heat environment" was conducted at Research Farm & laboratory of Department of Plant Physiology (Division of Basic Science), Indian Institute of Pulses Research (IIPR), Kalyanpur, Kanpur, Uttar Pradesh. During the Rabi season 2015-16. The chickpea (*Cicer arietinum* L.) various physiological traits which are essential for its adaptation to drought or water limiting environments. Among those, osmotic adjustment is considered to be one of the important traits conferring tolerance to terminal drought. The present study is aimed to investigate the role of osmotic adjustment in improving the water relation characteristics and maintaining photosynthesis under severe water stress. The 10 advanced breeding lines (ABLs) [C-7, C-8, C-9(M-51), C-11(M-86), C-16(M-55), C-19(M-93), C-20(M-39), C-21, C-214, C-235] derived from high and low osmotic adjustment (OA) were used for the experiments. For the purpose we measured reproductive stage (pollen viability) and yield attribute parameters (total biomass, pod numbers, seed yield and harvest index) which were used to determine drought and high temperature effects on chickpea. The pollen viability was tested by using 1% TTC solution. The results indicated that, a genetic difference exists in pollen viability at higher temperature in chickpea. At extreme high temperature 45 °C, only few lines such as C7, C8 and Tyson showed viable pollens. These genotypes were also identified as drought tolerant based upon osmolytes accumulation. The significant variation in yield attributing traits was observed among ABLs depending upon their ability of drought tolerance by means of osmolyte accumulation. The known drought tolerant genotypes Tyson and some of its derived ABLs showed improved yield in terms of total biomass, pod numbers, seed yield and harvest index as compared to drought sensitive lines DCP 92-3 and Kaniva.

Keywords: Osmotic adjustment, osmolytes, pollen viability, harvest index

1. Introduction
Chickpea (*Cicer arietinum* L.) is an important protein-rich cool-season food legume crop. Because it is cultivated predominantly in rainfed environments, it usually faces terminal drought during pod and seed filling, leading to significant reductions in grain yield (Siddique *et al*., 2000) [35]. Previous work has shown that earliness and phenological plasticity are important traits that result in high yields under the water-limited environments in which chickpea is grown (Berger *et al*., 2004, 2006) [40, 41]. However, additional drought-avoidance and drought tolerance traits have been suggested as benefiting yield in water-limited environments (Turner, 1997) [42]. Among the various traits, osmotic adjustment (OA) is considered an important physiological trait for adaptation to drought (Turner & Jones, 1980) [30]. The capacity for OA is relatively high in chickpea and has been reported to range from 0 to 1.3 MPa among chickpea cultivars (Morgan *et al*., 1991; Lecoeur *et al*., 1992, Leport *et al*., 1999; Moinuddin & Khanna-Chopra, 2004) [25, 18, 19, 26]. OA in plant cells maintains cell turgor, which in turn maintains physiological processes such as stomatal opening, photosynthesis, expansion growth (Turner & Jones, 1980; Ludlow & Muchow, 1990; Blum, 1996) [30, 20], delays senescence and death (Flower and Ludlow, 1986) [12], reduces flower abortion (Morgan & King, 1984) and improves root growth and ater extraction from the soil as water deficits develop (Morgan & Condon, 1986) [27].

In pigeonpea (*Cajanus cajan*), high OA led to improved leaf area duration, increased radiation use efficiency, higher growth rates and improved dry matter production under drought (Subbarao *et al*., 2000) [37].

However, not all observations of OA have shown positive physiological benefits. For example, while Jones & Rawson (1979) [16] reported that OA maintained higher rates of photosynthesis at low turgor in sorghum (Sorghum bicolor), Leport *et al*. (1999) [17] observed that photosynthesis was not maintained at high rates as leaf water potential (WP) decreased in chickpea. Moreover, in chickpea, OA was reversed during seed filling (Leport *et al*., 1999) [17], presumably as assimilates were preferentially mobilized to the seed, and recycling of respired carbon within the pod has been suggested as a means of maintaining seed filling when leaf photosynthesis is low (Turner, 2003; Furbank *et al*., 2004; Turner *et al*., 2005) [28, 13, 29]. Furthermore, chickpea often experiences terminal heat during reproductive face due to climate change. Drought and heat are the most important constraints to chickpea production globally. It is estimated that drought and heat stresses together account for about 50% of the yield losses caused by abiotic stresses. The economic value of these losses is estimated at US$ 1.28 billion. Chickpea is a dry and cool season crop, largely grown rainfed on residual soil moisture after the rainy season. The progressively receding soil moisture conditions often lead to moisture stress towards end of the crop season (terminal drought) causing heavy yield losses. Development of cultivars that can escape (early maturity) or tolerate (greater extraction of water from the soil, and enhanced water use efficiency) terminal drought is a major objective in chickpea breeding program (Gaur *et al*., 2012; Varshney *et al*., 2011) [14, 39].

Exposure to heat stress (≥ 35 ºC) at flowering and podding in chickpea is known to result in drastic reductions in seed yields (Summerfield *et al*., 1984; Devasirvatham *et al*., 2012) [38, 10]. In comparison to drought and other abiotic stresses, heat stress has received relatively less attention in chickpea breeding programs in the past. However, it has received considerable attention during the recent years. It is now well recognized that heat stress at the reproductive stage is increasingly becoming a serious constraint to chickpea productivity. This is because of: (i) large shift in chickpea area from cooler long-season environments to warmer short-season environments, (ii) increasing chickpea area under late sown conditions due to increasing cropping intensity, and (iii) expected overall increase in temperatures due to climate change (Gaur *et al*. 2012) [14]. In India, the chickpea area reduced by 4.3 million ha (from 5.1 million ha to 0.8 million ha) in northern and north-western India (Punjab, Haryana, Uttar Pradesh, Punjab), which has cooler long-season environments, and increased by 4.3 million ha (from 2.0 million ha to 6.3 million ha) in central and southern India (Madhya Pradesh, Maharashtra, Andhra Pradesh, Karnataka), which has relatively warmer and short-season environments. Thus, there has been a considerable increase in chickpea cultivation in areas which are prone to heat stress during reproductive development.

2. Materials and Methods

The present investigations were conducted using 9 advanced breeding lines namely C-7, C-8, C-9 (M-51), C-11 (M-86), C-16 (M-55), C-19 (M-93), C-20 (M-39), C-21, C-23 (M-10), derived from crosses chickpea (*Cicer arietinum* L.) genotypes Tyson x Kaniva along with 5 checks including known drought tolerant (Tyson), drought sensitive (Kaniva) and heat tolerant (JG 14) and sensitive (DCP 92-3), C-214, and C-235 chickpea genotypes. Experimental material 2-way crosses were made using chickpea genotypes with low OA (Kaniva) while another set having medium OA (Tyson) crossed with low OA (Kaniva). The populations were advanced to F6 stage and 10 advance breeding chickpea lines at F6 stage are to be evaluated under rainfed for osmotic adjustment and other physiological traits along with high yielding varieties DCP 92-3 (heat sensitive) and JG 14 (heat tolerant) as checks both of which are lacking osmotic adjustment. The trial was conducted under rainfed with progressive receding soil moisture conditions. The drought under field conditions as well as under laboratory situation was simulated either by withholding irrigation or by using polyethylene glycol 6000.

2.1 Experimental site and Location

The experiment was conducted at Research Farm & laboratory of Department of Plant Physiology (Division of Basic Science), Indian Institute of Pulses Research (IIPR), Kalyanpur, Kanpur and Uttar Pradesh.

2.2 Pollen viability test

In-vitro pollen fertility at high temperature in relation to osmotic adjustment was evaluated when crops exposed to heat stress. *In- vitro* pollen fertility was assessed by 2, 3, 5-triphenyl Tetrazolium chloride (TTC) tests (Hauser and Morrison, 1964) [43].

Plant Materials and Heat treatment

1. Unopened flower buds, Microscope. Incubator.
2. Micro pipette, Slide, cover slip & forceps.
3. 2, 3, 5-triphenyl tetrazolium chloride.

Evaluation of *In-vitro* pollen fertility at high temperature for Excised unopened flower bud of different genotypes were carried out by putting flower in Petri dishes with two layers of moistened filter papers. The Petri dishes were put in incubators at 30 °C, 35 °C, 40 °C and 45 °C temperature for 2 hours.

Experimental Method

After 2 hours, after acclimation and high temperature treatment, the pollen viability tests were tested individually for each plant type. TTC (2.3.5-triphenyl tetrazolium chloride) stain tests used for this purpose. A few drops of 1% TTC (0.2%g. TTC and 12g; sucrose were dissolved in 20 ml water) were dropped by Pasteur pipettes on microscope slide and pollen were shacked with a slim brush (each brush used only one plant type) covered with a cover slip. 3 different areas of each cover slip of used four microscope slides with three replication were counted within a 2 hours for TTC tests. Viable pollens were dyed in red and light red; dead pollens were not dyed in TTC.

2.3 Assessment of yield attributing traits

Total biomass per plant (g)

The completely matured five plants were uprooted carefully along with roots and were dried completely. The weight of dried plant along with pods was recorded as biological yield in grams.

Number of pods per plant

Total number of seed bearing pods on each plant was counted on five randomly selected plants at maturity and the average was recorded.

Number of seeds per plant

The number of seeds per plant was counted from taken all pods on five randomly selected plants at maturity.

Seed yield per plant (g)

The seed weight in grams from five randomly selected plants was recorded.

100 seed weight (g)

Hundred seeds were counted randomly from each genotype and the test weight was recorded in grams.

Harvest index:

The harvest index is expressed as the percent ratio between the economic yield and total biological yield and was suggested by Nichiporovich (1951).

$$HI = \frac{\text{Economic yield}}{\text{Total biological yield}} \times 100$$

3. Results and Discussion

3.1 Pollen viability

The pollen viability was tested by using 1% TTC solution. The viable pollens were identified microscopically with deep purple colour stained bodies while faint or diffuse stain indicated the sterile pollens. The viability decreased in chickpea genotypes with increased period of DAE to high temperature (> 30 °C, 35 °C, 40 °C, 45 °C) as indicated by loss of purple stain. (Fig 3.1-3.5).

The pollen viability decreased significantly as temperature increased. At high temperature above 35 °C loss of purple stain indicated decrease in pollen viability usually occurred in sensitive genotypes of chickpea. Less than 50% pollen viability was observed at temperature 40 °C in all the heat sensitive lines of chickpea while 50-60% pollen viability noticed in tolerant ones. All the test lines showed partial or complete pollen viability upto temperature 40^0C. Reduced or lack of pollen viability were the characteristic effects of high temperatures. Generally, pollen treated at high temperature did not take stain either in acetocarmine solution or 1% TTC solution indicated the pollen sterility induced by high temperature. At higher temperature (45 °C), only few pollen remained viable and often completely sterile in heat sensitive (HS) lines. The results indicated that, a genetic difference exists in pollen viability at higher temperature in chickpea. At extreme high temperature 45 °C, only few lines such as C7, C8 and Tyson showed viable pollens. These genotypes were also identified as drought tolerant based upon osmolytes accumulation. A typical example of non-viable and viable pollens of variety C214 has been shown in Fig 3.4 and 3.5.

All plant processes are irreversibly damaged by heat if plants are subjected to sufficiently hot temperatures for sufficient long durations. Several studies demonstrated that crop species are sensitive to heat during reproductive development. Because the fruit or the seed often are the economic product, these heat stress effects can substantially influence productivity. Different stages of reproductive development can be damaged by hot weather, including floral bud development, seed and fruit set, and embryo, seed, and fruit development. Pollen development and/or transfer are particularly sensitive to heat stress. In most cases, high night temperature was more damaging than high day temperature, and most sensitive development stage occurred just after meiosis and at the release of the microspores from the tetrad.

Apparently, pollen development represents a weak link in the ability of plants to withstand to high temperatures. Ahmed *et al*. (1992) [44] reported that tapetal cells (meiosis II) did not become binucleate and the locular cavity was less developed in anthers under high temperatures (33 °C day/ 30 °C night) resulting in premature pollen development. Such information is lacking in chickpea at meiosis stage.

Most of the pollen studies in chickpea have focused on cold tolerance (Srinivasan *et al*. 1999; Clarke *et al*. 2004) [45, 46] and the meiosis stage (9 days to 5-6 days before anthesis (DBA) of chickpea was found to be sensitive to cold (< 3 ºC). (Siddhique 2004) [47], Porch and Jahn (2001) [48] studied that high temperature effects on pre-anthesis are related to another development, pollen sterility and pollen production. The study of pollen may help to predict genetic variation among genotypes for reproductive phase heat tolerance. Pollen sterility is one of the key factors limiting legume yield under high temperature.

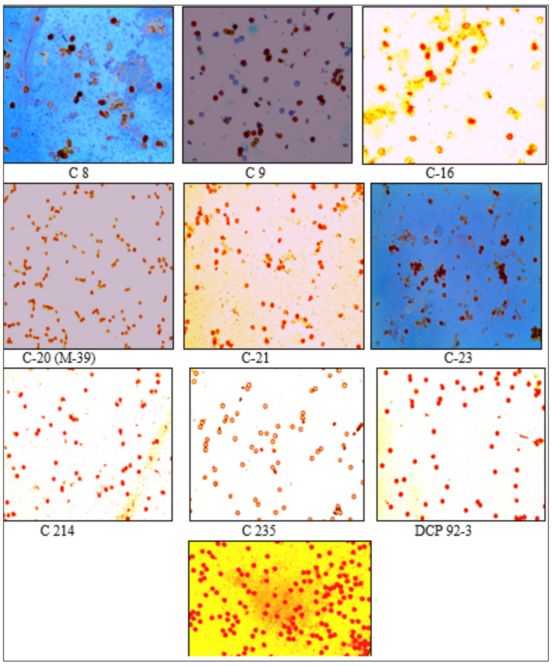

Fig 3.1: Pollen viability of chickpea genotypes at 30^0C

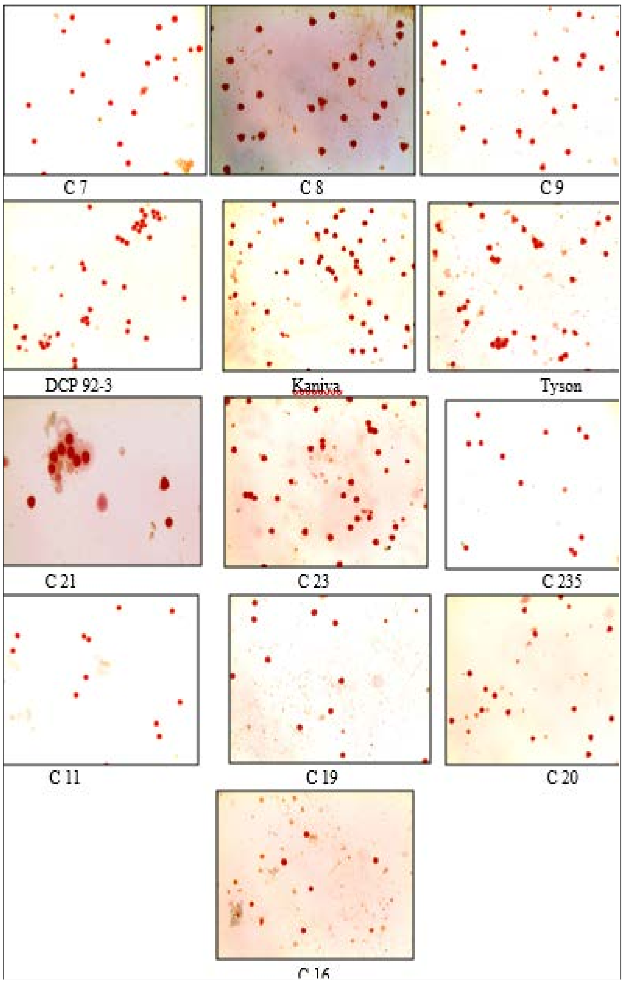

Fig 3.2: Pollen viability of chickpea genotypes at 35 °C

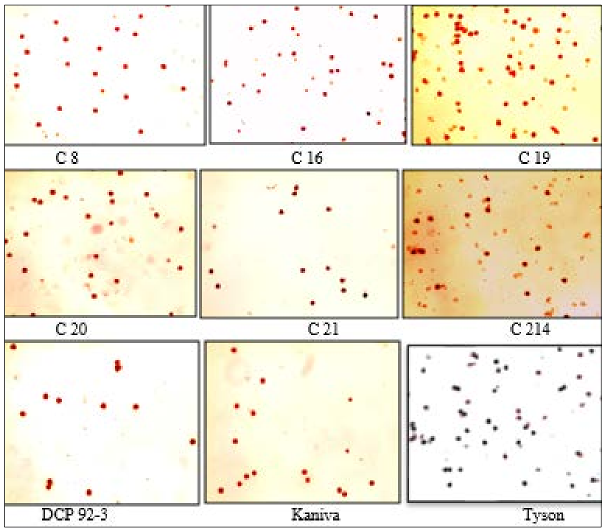

Fig 3.3: Pollen viability of chickpea genotypes at 40^{0}C

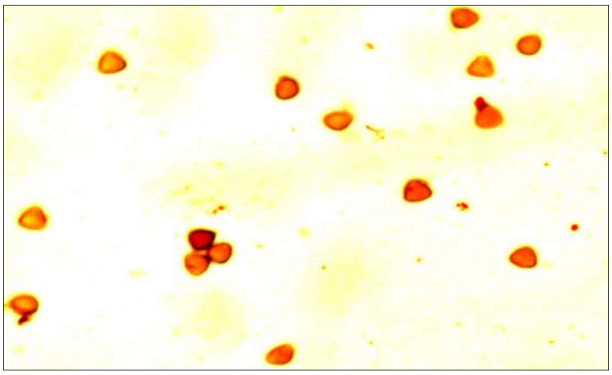

Fig 3.4: C 214, sterile pollens at 45 °C

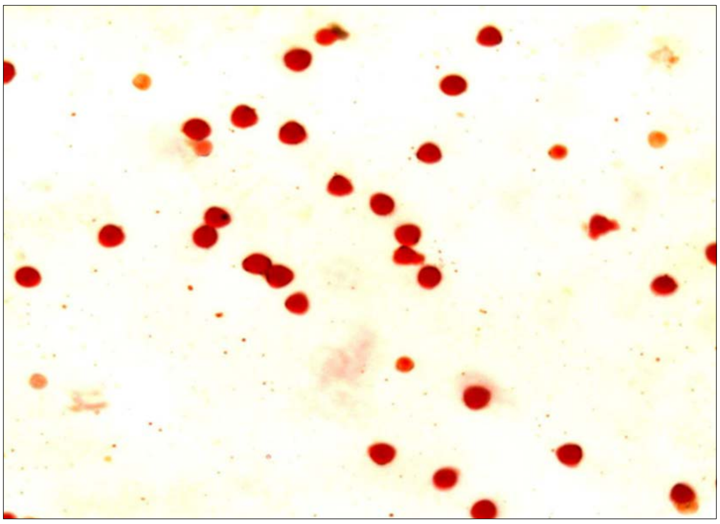

Fig 3.5: C 214 (Fertile pollens/ at 35 °C

3.2 Yield and its attributes of genotypes

Data representing in Table 3.1. The known drought tolerant genotypes Tyson and some of its derived ABLs showed improved yield in terms of total biomass, pod numbers, seed yield and harvest index as compared to drought sensitive lines DCP 92-3 and Kaniva. The significant variation was observed in yield attributing traits was observed among ABLs depending upon their ability of drought tolerance by means of osmolyte accumulation, sugar and proline accumulation, photosynthetic efficiency and also partitioning efficiency. The accumulation of low molecular weight compounds during water stress such as sugars, amino acids, proline, organic acids etc facilitate the photosynthate transport as evidenced by increase in the harvest index as well as these compounds also impart tolerance to drought as they are osmo protectant substances that prevent membrane disorganization and also maintain leaf turgor. Among various trait, osmotic adjustment (OA) is an important physiological trait for adaptation to drought (Turner and Jones, 1980) [30].

The capacity for OA is relatively high in chickpea and has been reported to range from 0 to 1.3 MPa among chickpea cultivars (Morgan *et al* 1991; Lecoeur *et al*., 1992; Leport *et al*., 1999; Moinuddin and Khanna Chopra 2004) [25, 18, 19, 26]. OA in plant cells maintains cell turgor, which turn maintains physiological processes such as stomatal opening, photosynthesis, expansion growth and water extraction (Ludlow and Muchow, 1990) [20], reduces flower abortion (Morgan and King, 1984) and improve root growth and water extraction from soil as water deficit develops (Morgan and Condon, 1986) [27]. In pigeon pea, high OA led to improved leaf area duration, increase radiation use efficiency, higher growth rates and increase dry matter production under drought (Subbarao *et al*., 2000) [37].

Table 3.1: Yield attributes of the chickpea genotypes grown under rainfed condition

Genotype	Total Biomass	Number of total pod	No. of seed	Seed yield per plant (g)	Harvest index (%)	100 seed wt
TYSON	58.3	190	238.7	35.3	60.733	13.3
DCP-92-3	64.2	201	250.3	28.8	42.067	15.6
KANIVA	66.7	104	103	20.3	22.467	36.1
C-7	68	102	110.7	38.4	56.433	36.8
C-8	73.8	110	152	39.3	48.833	36.1
C-9 (M-51)	40.9	137	138	31.4	43.867	24.6
C-11 (M-86)	38.2	78	114	16.6	41.933	21.1
C-16 (M-55)	44.4	172	206.3	27.2	61.433	14.4
C-19 (M-93)	45.6	132	136	26.8	60.9	15.6
C-20 (M-39)	48.9	154	159	31.5	65.433	18.4
C-21	46.7	143	146.7	22.9	47.133	17.4
C-23(M-10)	41.7	70	112.7	14.6	32.9	17.3
C-214	49.9	140	146.7	24.3	46.933	14.1
C-235	41.9	172	175.3	22.5	53.633	15.4
C.D.	2.7	4.539	4.054	3.5	4.286	
SE(m)	0.9	1.553	1.387	1.2	1.466	
SE(d)	1.3	2.196	1.961	1.7	2.074	
C.V.	3.1	1.878	1.469	7.2	4.907	

4. Conclusions

The trials were conducted under rainfed with progressive receding soil moisture conditions. The drought under field conditions as well as under laboratory situation was simulated either by withholding irrigation or by using polyethylene glycol 6000.

1. For the purpose we measured pollen viability & yield attribute parameters which were used to determine drought and high temperature effects on chickpea and understand the role of OA. The results obtained are pollen viability was tested by using 1% TTC solution. The results indicated that, a genetic difference exists in pollen viability at higher temperature in chickpea. At extreme high temperature 40 °C, only few lines such as C 7, C8 and Tyson showed viable pollens. These genotypes were also identified as drought tolerant based upon osmolytes accumulation.
2. The significant variation in yield attributing traits was observed among ABLs depending upon their ability of drought tolerance by means of osmolyte accumulation. The known drought tolerant genotypes Tyson and some of its derived ABLs showed improved yield in terms of total biomass, pod numbers, seed yield and harvest index as compared to drought sensitive lines DCP 92-3 and Kaniva.

5. Reference

1. Basu PS, Singh DN. Physiology and abiotic stress in chickpea. In: M. Ali, S. Kumar, and N. B. Singh (ed), Chickpea Research in India, 137-167. Indian Institute of Pulses Research, Kanpur, India; c2003.
2. Basu PS, Berger JD, Turner NC, Chaturvedi SK, Ali M, Siddique KHM. Osmotic adjustment of chickpea (*Cicer arietinum*) is not associated with changes in carbohydrate composition or leaf gas exchange under drought. Ann. Appl. Biol. 2007;150:217-225.
3. Basu PS, Ashoo S, Sukumaran NP. Changes in net photosynthetic rate and chlorophyll fluorescence in potato leaves induced by water stress. Photosynthetica. 1998;35:13-19.
4. Basu PS, Berger JD, urner NC, Chaturvedi SK, Ali M, Siddique KHM. Osmotic adjustment of chickpea (*Cicer arietinum*) is not associated with changes in carbohydrate composition or leaf gas exchange under drought. Annals of Applied Biology. 2007;150:217-225.
5. Basu PS, Ali, Masood, Chaturvedi SK. Osmotic adjustment increases water uptake, remobilization of assimilates and maintains photosynthesis in chickpea under drought. Indian Journal of Experimental Biology (CSIR). 2007;45:261-267.
6. Basu PS, Ali M, Chaturvedi SK. Terminal heat stress adversely affects chickpea productivity in Northern India-Strategies to improve thermo tolerance in the crop under climate change. In: ISPRS Archives XXXVIII-8/W3 Workshop Proceedings: Impact of Climate Change on Agriculture, New Delhi, India; c2009 Feb. p. 23-25.
7. Cornic G, Fresneau C. Photosynthetic carbon reduction and carbon oxidation cycles are the main electron sinks for photosystem 2 activity during a mild drought. Annals Bot. 2002;89:887-894.
8. Conroy JP, Smillie M, Kuppers D, Bevege, Barlow DI. Chlorophyll a fluorescence and photosynthetic and growth responses of *Pinus radiata* to phosphorus deficiency, drought stress and high CO_2. Plant Physiology; c1986.
9. Dimarco G, Massaci A, Gabrielli R. Drought effects on photosynthesis and fluorescence in hard wheat cultivars grown in the field. Physiologia Planterum. 1988;74:385-390.
10. Devasirvatham V, Gaur PM, Mallikarjuna N, Tokachichu RN, Trethowan RM, Tan DKY. Effect of high temperature on the reproductive development of chickpea genotypes under controlled environments. Functional Plant Biology. 2012;39:1009-1018.
11. Devasirvatham V, Tan DKY, Gaur PM, Raju TN, Trelhowan RM. High temperature tolerance in chickpea

and its implications for plant improvement. Crop 'Pasture Sci. 2012c;63:419-428.
12. Flower DJ, Ludlow MM. Contribution of osmotic adjustment to the dehydration tolerance of water-stressed pigeon pea (*Cajanus Cajan* (L.) Millsp.) Leaves. Plant, Cell and Environment. 1986;9:33-40.
13. Furbank RT, White R, Palta JA, Turner NC. Internal recycling of respiratory CO_2 in pods of chickpea (*Cicer Arietinum* L.): The role of pod wall, seed coat, and embryo. Journal of Experimental Botany. 2004;55:1687-1696.
14. Gaur PM, Jukanti AK, Srinivasan S, Gowda CLL. Chickpea *(Cicera ridinum* L.), in Breeding of Field Crops (ed. D.N. Bharadwaj), Agrobios (India), Jodhpur; c2012. p. 165-194.
15. Genty B, Briantais JM, Baker JM. The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. Biochimica ET Biophysica Acta. 1989;990:87-92.
16. Jones MM, Rawson HM. Influence of the rate of development of leaf water deficits upon photosynthesis, leaf conductance, water use efficiency, and osmotic potential in sorghum. Physiologia Plantarum. 1979;45:103-111.
17. Leport L, Turner NC, French RJ, Barr MD, Duda R, Davies SL, *et al*. Physiological response of chickpea genotype to terminal drought in a Mediterranean environment. European Journal of Agronomy. 1999;11:279-291.
18. Lecoeur J, Wery J, Turc O. Osmotic adjustment as a mechanism of dehydration postponement in chickpea (*Cicer arietinum* L.) leaves. Plant and Soil. 1992;144:177-189.
19. Leport L, Turner NC, French RJ, Barr MD, Duda R, Davies SL, *et al*. Physiological responses of chickpea genotypes to terminal drought in a Mediterranean-type environment. European Journal of Agronomy. 1999;11:279-291.
20. Ludlow MM, Muchow RC. A critical evaluation of traits for improving crop yields in water-limited environments. Advances in Agronomy. 1990;43:107-153.
21. Ludlow MM. Contribution of osmotic adjustment to the maintenance of photosynthesis during water stress. In Progress in Photosynthesis Research (J. Biggins, Ed.). 1987;4:161-168.
22. Maxwell K, Johnson GN. Chlorophyll fluorescence- a practical guide. J Exp. Bot. 2000;51:659-668.
23. Morgan JM. Increases in grain yield of wheat by breeding for an osmo regulation gene: relationship to water supply and evaporative demand. Aust. J. Agric. Res. 2000;51:971-978.
24. Morgan JM. Osmo regulation and water stress in higher plants. Annu. Rev. Plant Physiol. 1984;35:299-319.
25. Morgan JM, Rodriguezmaribona B, Knights EJ. Adaptation to water-deficit in chickpea breeding lines by osmo regulation-relationship to grain yields in the field. Field Crops Res. 1991;27:61-70.
26. Moinuddin, Khanna-Chopra R. Osmotic adjustment in chickpea in relation to seed yield and yield parameters. Crop Sci. 2004;44:449-455.
27. Morgan JM, Condon AG. Water use, grain yield and osmo regulation in wheat. Australian Journal of Plant Physiology. 1986;13:523-532.
28. Turner NC. Adaptation to drought: lessons from studies with chickpea. Indian Journal of Plant Physiology Special; c2003. p. 11-17.
29. Turner NC, Davies SL, Plummer JA, Siddique KHM. Seed filling in grain legumes under water deficits with emphasis on chickpea (*Cicer arietinum* L.). Advances in Agronomy. 2005;87:211-250.
30. Turner NC, Jones MM. Turgor maintenance by osmotic adjustment. In: Turner NC, Kramer PJ, eds. Adaptation of plants to water and high temperature stress. New York: Wiley; c1980. p. 87-103.
31. Schreiber U. Pulse-amplitude (PAM) fluorometry and saturation pulse method. In: Papageorgiou G, Govindjee (eds) Chlorophyll fluorescence: A signature of Photosynthesis. Kluwer, Dordrecht; c2004. p. 279-319.
32. Schreiber U, Bilger W, Schliwa U. Continuous recording of photochemical and non-photochemical chlorophyll fluorescence quenching with a new type of modulation fluorometer. Photosynth Res. 1986;10:51-62.
33. Serraj R, Sinclair TR. Osmolyte accumulation: can it really help increase crop yield under drought conditions? Plant, Cell and Environment. 2002;25:333-341.
34. Serraj, Sinclair. Concluded that no yield advantage from OA had been demonstrated in any crop except at very low and uneconomic yields; c2002.
35. Siddique KHM, Brinsmead RB, Knight R, Knights EJ, Paul JG, Rose IA. Adaptation of chickpea (*Cicer arietinum* L.) and faba bean (*Vicia faba* L.) to Australia. In Linking Research and Marketing Opportunities for Pulses in the 21st Century. Ed R. Knight. Dordrecht, the Netherlands: Kluwer; c2000. p. 289-303.
36. Siddique MRB, Hamid A, Islam MS. Drought stress effects on water relations of wheat. Botanical Bulletin of Academia Sinica. 2000;41:35-39.
37. Subbarao GV, Johansen C, Slinkard AE, Rao RCN, Saxena NP, Chauhan YS. Strategies for improving drought resistance in grain legumes. Critical Reviews in Plant Sciences. 1995;14:469-523.
38. Summerfield RJ, Hadley P, Roberts EH, Min chin FR, Rawsthrone S. Sensitivity of chickpea (*Cicer arietinum* L.) to hot temperatures du ring the reproductive period. Exp. Agiic. 1984;20:77-93.
39. Varshney RK, Lekha P. Kashiwagi J, Gaur PM, Krishnamurthy L, Hoisington D. Genomics and physiological approaches for root trait breeding lo improve drought tolerance in chickpea (*Cicer arietinum* L.), in *Root Genomics* (eds AC De Oliveira and RK Varshney), Springer, Berlin. 2011;23:3-2.
40. Michalik L, Auwerx J, Berger JP, Chatterjee VK, Glass CK, Gonzalez FJ, *et al.* International Union of Pharmacology. LXI. Peroxisome proliferator-activated receptors. Pharmacological reviews. 2006 Dec 1;58(4):726-41.
41. Berger AN, Demirgüç-Kunt A, Levine R, Haubrich JG. Bank concentration and competition: An evolution in the making. Journal of Money, credit and Banking. 2004 Jun 1. p. 433-51.
42. Ellen IG, Turner MA. Does neighborhood matter? Assessing recent evidence. Housing Policy Debate. 1997 Jan 1;8(4):833-66.
43. Hauser EJ, Morrison JH. The cytochemical reduction of nitro blue tetrazolium as an index of pollen viability. American Journal of Botany. 1964 Aug;51(7):748-52.

44. Musavi MT, Ahmed W, Chan KH, Faris KB, Hummels DM. On the training of radial basis function classifiers. Neural networks. 1992 Jul 1;5(4):595-603.
45. Freedman DS, Dietz WH, Srinivasan SR, Berenson GS. The relation of overweight to cardiovascular risk factors among children and adolescents: the Bogalusa Heart Study. Pediatrics. 1999 Jun 1;103(6):1175-82.
46. Clarke HJ, Khan TN, Siddique KH. Pollen selection for chilling tolerance at hybridisation leads to improved chickpea cultivars. Euphytica. 2004 Mar;139:65-74.
47. Siddique R, Naik TR. Properties of concrete containing scrap-tire rubber: An overview. Waste management. 2004 Jan 1;24(6):563-9.
48. Porch TG, Jahn M. Effects of high-temperature stress on microsporogenesis in heat-sensitive and heat-tolerant genotypes of Phaseolus vulgaris. Plant, Cell & Environment. 2001 Jul;24(7):723-31.