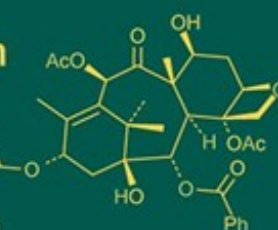
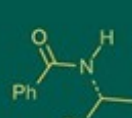
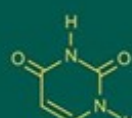


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Studies on osmolytes accumulations, membrane stability, antioxidants defence system, seed yield and pollen viability in chickpea (*Cicer arietinum* L.) under different temperature stress in North West India

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ABSTRACT

An experiment was conducted in the fields of Pulses Section, Chaudhary Charan Singh Haryana Agricultural University, Hisar, Haryana during Rabi seasons of 2017-18 and 2018-19 under different sowing dates. Temperature stress (High or Low) was imposing by manipulating the sowing dates that is 15th October (early sown condition in Haryana), 15th November (Normal sown condition in Haryana) and 15th December (late sown condition in Haryana). Data were recorded when temperature was < 50C in early sown crops while > 350C in late sown condition at 50 % flowering stage (80-90 DAS) to investigate the osmolytes accumulations, membrane stability in terms of MDA content and H₂O₂ production, seed yield and antioxidants defence system adopted by chickpea genotypes under high and low temperature stress. Results indicated that early sown crops experienced low temperature stress in the month of December-January while late sown crops showed high temperature stress in the month of March- April. The osmolytes accumulations and antioxidant activities are maximum in 15th December sowing followed by 15th October while minimum in 15th November sowing. The membrane stability and pollen viability was maximum in 15th November sowing followed by 15th October and minimum was found in 15th December sowing. Among genotypes maximum osmolytes accumulations, antioxidant activities, membrane stability and pollen viability were observed in H12-64 and H13-01 while minimum were found in H14-04 and no significant differences in seed yield was recorded between 15th October and 15th November sowing, however, further delay in sowing to 15th December showed significant reduction in seed yield.

Keywords: Antioxidants, Chickpea, Oxidative Stress, Sowing dates, Temperature

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Introduction

In India, Madhya Pradesh, Rajasthan, Maharashtra, Uttar Pradesh, Andhra Pradesh, Karnataka, Chhattisgarh, Bihar and Jharkhand are major chickpea cultivating and producing states contributing more than 95% to the total chickpea area and production. Madhya Pradesh is the single largest producer in the country accounting for over 41% of total production while Maharashtra, Rajasthan, Karnatak, Uttar Pradesh and Andhra Pradesh contribute about 16%, 13%, 8%, 6% and 6% while area contribute about 34%, 19%, 17%, 13%, 6% and 5%, respectively (Fig. 1.1).

In recent years, India has made remarkable progress in expanding chickpea area and production and the area under cultivation for chickpea has been increased from about 5.19 m ha in 2000-01 to 10.76 m ha in 2017-18. This is coupled with increase in production (3.86 mt in 2000-01 to 11.1 mt in 2017-18) and productivity (7.44 q/ha in 2000-01 to 10.31 q/ha in 2017-18) of chickpea as well (Fig. 1.2).

Sowing time can influence different climatic parameters, such as temperature, moisture, sunlight, etc. affecting plant growth (Bazvand *et al.*, 2015) [1]. In northern part of India, it is normally sown during second fortnight of October. Sometime its sowing is delayed depending upon the withdrawal of monsoon and late harvest of preceding *khari* crop. Legumes are sensitive to variation in temperature and the late-sown crop is exposed to high temperatures (>35°C) at its reproductive stage in the months of February and March while early sown crops experienced low temperature in the month of December-January (Berger *et al.*, 2011; Kumar *et al.*, 2012) [2, 3].

High temperature can affect many physiological processes into plants like photosynthesis, respiration, membrane stability and water status that results into increased concentrations of a variety of heat shock proteins (HSPs) and production of reactive oxygen species (Wahid *et al.*, 2007) [4] leading to poor seed set (Young *et*

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et al., 2004) [5]. However, during early sown conditions, temperature below 10 °C is known to alter a variety of physiological processes ranging from plant water status, photosynthesis to reactive oxygen species (ROS) and solute accumulation, excessive floral abortion and has adverse effect on chickpea yield and results in losses from 15-20 % (Ali and Kumar, 2005, Chaturvedi *et al.*, 2009) [6]. To nullify the damage due to oxidative stress plants have well developed several enzymatic and non-enzymatic antioxidant defense systems to avoid and detoxify the adverse effects of ROS which include the enzymes: catalase (CAT), peroxidase (POX), superoxide dismutase (SOD), ascorbate peroxidase (APX), glutathione reductase (GR) and antioxidants such as glutathione and ascorbate content as observed in chickpea (Kumar *et al.*, 2012) [3]. Under high temperature stress, plants are found to accumulate enhanced amount of non-enzymatic antioxidant and helps in up regulation of the specific activities of antioxidant enzymes. Superoxide dismutase (SOD) catalyzes the dismutation of superoxide into hydrogen peroxide and oxygen which is in-turn metabolized by the action of peroxidases (Ahmad *et al.*, 2008). CAT converts H₂O₂ to water and molecular oxygen. In plant, CAT is localized, predominantly in peroxisomes and glyoxysomes where it functions chiefly to remove the H₂O₂ formed during the process of photorespiration. POX localized in almost all compartments of the plant cell where, it plays role in stability the level of H₂O₂. Peroxidases, besides their main function in H₂O₂ elimination, can also catalyse O₂ and H₂O₂ formation by a complex reaction in which NADH is oxidized using trace amounts of H₂O₂. Peroxidases are also involved in biosynthesis of cell wall lignifications and suberization (Passardi *et al.*, 2004) [7]. There are numerous plant studies which indicate the tolerance to temperature stress in plants is accompanied with an increase in specific activities of antioxidants (Gill and Tuteja, 2010; Kaushal *et al.*, 2011; Hasanuzzaman *et al.*, 2012; Kumar *et al.*, 2012) [8, 9, 10, 3]. Therefore, recognizing the characterizations of plants cell responses during cold stress (CS) and heat stress will help us improve temperature tolerant development strategies.

Materials and methods

Hisar is located in North-West India at 215.2 meters above mean sea level with latitude of 29° 10' North and longitude of 75° 46' East. The minimum temperature in this area reaches upto 0.5 °C in December and January and the maximum temperature in the area reaches upto 48 °C during May or June. The mean weekly maximum temperature ranged between 16.9 to 39.3 °C and minimum between 2.6 to 20.6 °C, while, their corresponding values in 2018-19 were 17.1 to 40.7 °C and 1.9 to 20.6 °C respectively (Fig 1.1 and 1.2). Following methods were used for estimation of osmolytes and membrane stability as under:

1. Proline: Proline content was estimated by using the method of Bates *et al.* (1973) [11].
2. Glycine betaine: Glycine betaine was measured according to Grieve *et al.* (1983) [12]
3. Total Soluble Carbohydrates (TSC): Total soluble carbohydrates were determined with the method of Yemm *et al.* (1954) [13] using anthrone reagent.
4. H₂O₂ Content: H₂O₂ content of the leaves was determined by a modified Patterson *et al.* (1984) [14] method.
5. Malondialdehyde (MDA) Content: The level of lipid peroxidation was measured in terms of malondialdehyde (MDA) present in the leaves tissues MDA is a product of lipid peroxidation and was measured by thiobarbituric acid (TBA) reaction with minor modifications of the method of Heath and Packer (1968) [15].
6. Pollen viability (%)

Viability of freshly released pollen grains was assessed by 2,3,5 triphenyl tetrazolium chloride (TTC) test (Hauser *et al.*, 1964) [16]. 500 mg of leaves were crushed in 5 ml of 0.1 M potassium phosphate buffer (pH 7.0). The extract was then centrifuged at 10,000 at 4 °C for

20 minutes. The supernatant was then used for the estimation of the following enzymes:

- 1 **Superoxide dismutase (EC1.15.1.1)** SOD activity was estimated by the method of Beauchamp *et al.* (1971) [17].
- 2 **Catalase (EC 1.11.1.6)** Catalase (CAT) activity was estimated by the UV method of Aebi (1983) [18]
- 3 **Peroxidase (EC 1.11.1.7)** Peroxidase (POX) activity was estimated by the method of Shannon *et al.* (1966) [19].
- 4 **Ascorbate peroxidase (APX) (EC 1.11.1.11)** The enzyme activity was determined following the oxidation of ascorbic acid (Nakano *et al.*, 1981) [20].
- 5 **Glutathione reductase (EC 1.6.4.2)** Glutathione reductase activity was estimated by the method of Halliwell *et al.* (1978) [21].
- 6 **Ascorbate content** : Ascorbic acid content was determined with a modification of the procedure of Takahama *et al.* (1992) [22].
- 7 **Glutathione content**: Content of oxidized, reduced and total glutathione was estimated by the method of Smith *et al.* (1985) [23].

Statistical analysis

Data were subjected to pooled analysis of both year (2017-18 and 2018-19) and analysis of (ANOVA) using Online Statistical Analysis Package (OPSTAT) Computer Section, CCS Haryana Agricultural University, Hisar. Correlation of various parameters with seed yield were determined by using Pearson's correlation coefficient.

Results and discussion

Pollen viability (%)

In current study, the pollen viability in chickpea differed significantly at 50 % flowering stage. Among three dates of sowings lowest pollen viability (64.11 %) was recorded in crop sown on 15th December and highest (69.00 %) in November 15 sowing. This might be due to comparatively high temperature (28.9°C) prevailing at 50% flowering in 15th December sowing than 15th November sowing (22.2°C). The data in table 1.1 showed that highest (72.19 %) pollen viability in genotypes was recorded in genotype H13-01 whereas, minimum (60.47 %) pollen viability was in H14-04 this might be due to their different genetic make-up. Similar results due to temperature variation also observed (Canci and Toker, 2009; Krishnamurthy *et al.* 2011; Upadhyaya *et al.* 2011) [24, 25, 26].

Osmolytes and membrane stability parameters

In the current study increased trends of compatible solutes were observed with delayed sowing beyond 15th November. The data presented in figure 2.1, 2.2 and 2.3 showed that among three dates of sowings, maximum proline content (0.915 mg g⁻¹ DW), glycine betaine content (0.204 mg g⁻¹ DW) and total soluble carbohydrates (7.5 mg g⁻¹ DW) in leaf were recorded in crop sown on December 15th and minimum (0.581, 0.028, 4.5 mg g⁻¹ DW) respectively, were in November 15th sowing at 50% flowering. This variation might be due to variation in temperature *i.e* 28.9 °C in 15th December sowing while 22.2 °C in 15th November sowing at 50% flowering stages. The increment of 6.7 °C temperature might be responsible for more accumulation of proline content, glycine betaine content and total soluble carbohydrates (TSC) in 15th December sowing. Similar results due to temperature variation also reported earlier by chickpea (Kumar *et al.*, 2012; Kaushal *et al.*, 2011) [9], Brassica (Takeda *et al.*, 2000) [27], wheat (Ahmed and Hasan, 2011). Accumulation of osmolytes like proline, glycine betaines and sugars has been observed in many studies on plants exposed to abiotic stresses (Zuther *et al.*, 2007; Kempa *et al.*, 2008; Lugan *et al.*, 2010) [29, 28, 30]. In the current study, among genotypes maximum proline content (0.839 mg g⁻¹ DW), glycine betaine content (0.206 mg g⁻¹ DW) and total soluble carbohydrates (7.6 mg g⁻¹ DW) in leaf were recorded in H13-01 followed by in H12-64 and minimum (0.656, 0.077, 5.9 mg g⁻¹ DW) respectively, were recorded in H14-04 and this might be due to different genetic potential of genotypes towards temperature variations. Similar genotypic differences due to temperature variations also presented earlier by Kumar *et al.*, 2012; Kaushal *et al.*, 2011 [3] in chickpea.

Similarly like proline the amount of TSC also increased rapidly in response to the increasing high temperature, this result agree with the result of some researchers who indicated that drought (Mafakheri *et al.* 2011; Jebory, 2012) [32, 31] and salinity stress induce soluble carbohydrates accumulation in chickpea (Nandwal *et al.*, 2007; Kukreja *et al.*, 2010) [34, 33].

The elevation of temperature has been reported to disturb membrane integrity of cell by inducing changes in the structure, lipid composition and membrane proteins (Barghi *et al.*, 2013) [35]. The malondialdehyde (MDA) content was measured to determine the lipid peroxidation level because MDA is a by product of lipid peroxidation. The content of MDA and hydrogen peroxide (H_2O_2) has been considered as an indicator of oxidative injury (Moller *et al.*, 2007) [36]. In present investigation, the increase in oxidative stress due to MDA content and H_2O_2 content were observed in late sowing (15th December). In fig. 2.4 and 2.5 data showed that among three dates of sowings, maximum MDA content ($63.8 \mu\text{ moles g}^{-1}\text{ DW}$) and H_2O_2 content ($15.4 \mu\text{ moles g}^{-1}\text{ DW}$) in leaf were recorded in crop sown on December 15th and minimum MDA content ($42.0 \mu\text{ moles g}^{-1}\text{ DW}$) and H_2O_2 content ($10.9 \mu\text{ moles g}^{-1}\text{ DW}$) in leaf were observed in November 15th sowing at 50% flowering stages. This variation might be due to variation in temperature *i.e* 28.9°C in 15th December sowing while 22.2 °C in 15th November sowing at 50% flowering stages. The increment of 6.7 °C temperature might be responsible for higher values of MDA content and H_2O_2 content in 15th December sowing. Similar results due to temperature variation also reported earlier by (Kaushal *et al.*, 2011; Kumar *et al.*, 2012 in chickpea; Savicka *et al.*, 2012 in wheat) [9, 3, 37]. Among genotypes minimum MDA content ($45.9 \mu\text{ moles MDA g}^{-1}\text{ DW}$) and H_2O_2 content ($6.9 \mu\text{ moles g}^{-1}\text{ DW}$) in leaf were recorded in H13-01 followed by in H12-64 and maximum MDA content ($57.8 \mu\text{ moles MDA g}^{-1}\text{ DW}$) and H_2O_2 content ($16.3 \mu\text{ moles g}^{-1}\text{ DW}$) were recorded in H14-04. Similar results due to temperature variation also reported earlier by (Kaushal *et al.* 2011; Kumar *et al.* 2012) [9] in chickpea.

Antioxidants parameters

In the present investigation, increased specific activity of enzymes (SOD, CAT, POX, APX, GR) along with increased accumulation of antioxidants (Ascorbate content and glutathione content) with delayed sowing (15th December) at 50% flowering stage. In fig. 2.6, 2.7, 2.8, 2.9, 2.10, 2.11 and 2.12. In fig. 2.6 data showed that among three dates of sowings, maximum specific activity of SOD ($6.7 \text{ Units mg}^{-1} (\text{protein}) \text{ min}^{-1}$) in leaf were recorded in crop sown on December 15th followed by 15th October ($2.2 \text{ Units mg}^{-1} (\text{protein}) \text{ min}^{-1}$) and minimum ($2.2 \text{ Units mg}^{-1} (\text{protein}) \text{ min}^{-1}$) were observed in November 15th sowing at 50% flowering stages. This might be accounted by variation in temperature as high temperature in 15th December sowing and low temperature in 15th October sowing causes more accumulation of compatible solutes (Proline, glycine betaines and TSC) that might be responsible for stabilization and maintenance the structure of these enzymes that ultimately resulted into increased specific activities of enzymes in 15th December and 15th October sowing than 15th November sowing. Similar results also reported earlier through various studies in different plants by (Gill and Tuteja, 2010 [8]; in wheat and Kaushal *et al.*, 2011 [9]; Hasanuzzaman *et al.*, 2012 [10]; Kumar *et al.*, 2012 [3] in chickpea). In fig. 2.7 data showed that among three dates of sowings, maximum specific activity of CAT ($7.0 \text{ Units mg}^{-1} (\text{protein}) \text{ min}^{-1}$) in leaf were recorded in crop sown on December 15th followed by 15th October ($2.2 \text{ Units mg}^{-1} (\text{protein}) \text{ min}^{-1}$) and minimum ($2.0 \text{ Units mg}^{-1} (\text{protein}) \text{ min}^{-1}$) were observed in November 15th sowing at 50% flowering stages. This might be accounted by variation in temperature as high temperature in 15th December sowing and low temperature in 15th October sowing causes more accumulation of compatible solutes (Proline, glycine betaines and TSC) that might be responsible for stabilization and maintenance the structure of these enzymes that ultimately resulted into increased specific activities of enzymes in 15th December and 15th October sowing than 15th November sowing. Similar results also re-

ported earlier through various studies in different plants by (Gill and Tuteja, 2010 [8]; in wheat and Kaushal *et al.*, 2011 [9]; Hasanuzzaman *et al.*, 2012 [10]; Kumar *et al.*, 2012 [3] in chickpea).

In fig. 2.8 data showed that among three dates of sowings, maximum specific activity of POX ($6.0 \text{ Units mg}^{-1} (\text{protein}) \text{ min}^{-1}$) in leaf were recorded in crop sown on December 15th followed by 15th October ($2.2 \text{ Units mg}^{-1} (\text{protein}) \text{ min}^{-1}$) and minimum ($2.7 \text{ Units mg}^{-1} (\text{protein}) \text{ min}^{-1}$) were observed in November 15th sowing at 50% flowering stages. This might be accounted by variation in temperature as high temperature in 15th December sowing and low temperature in 15th October sowing causes more accumulation of compatible solutes (Proline, glycine betaines and TSC) that might be responsible for stabilization and maintenance the structure of these enzymes that ultimately resulted into increased specific activities of enzymes in 15th December and 15th October sowing than 15th November sowing. Similar results also reported earlier through various studies in different plants by (Gill and Tuteja, 2010 [8]; in wheat and Kaushal *et al.*, 2011 [9]; Hasanuzzaman *et al.*, 2012 [10]; Kumar *et al.*, 2012 [3] in chickpea).

In fig. 2.9 data showed that among three dates of sowings, maximum specific activity of APX ($0.45 \text{ Units mg}^{-1} (\text{protein}) \text{ min}^{-1}$) in leaf were recorded in crop sown on December 15th followed by 15th October ($2.2 \text{ Units mg}^{-1} (\text{protein}) \text{ min}^{-1}$) and minimum ($0.23 \text{ Units mg}^{-1} (\text{protein}) \text{ min}^{-1}$) were observed in November 15th sowing at 50% flowering stages. This might be accounted by variation in temperature as high temperature in 15th December sowing and low temperature in 15th October sowing causes more accumulation of compatible solutes (Proline, glycine betaines and TSC) that might be responsible for stabilization and maintenance the structure of these enzymes that ultimately resulted into increased specific activities of enzymes in 15th December and 15th October sowing than 15th November sowing. Similar results also reported earlier through various studies in different plants by (Gill and Tuteja, 2010 [8]; in wheat and Kaushal *et al.*, 2011 [9]; Hasanuzzaman *et al.*, 2012 [10]; Kumar *et al.*, 2012 [3] in chickpea).

In fig. 2.10 data showed that among three dates of sowings, maximum specific activity of GR ($0.55 \text{ Units mg}^{-1} (\text{protein}) \text{ min}^{-1}$) in leaf were recorded in crop sown on December 15th followed by 15th October ($2.2 \text{ Units mg}^{-1} (\text{protein}) \text{ min}^{-1}$) and minimum ($0.23 \text{ Units mg}^{-1} (\text{protein}) \text{ min}^{-1}$) were observed in November 15th sowing at 50% flowering stages. This might be accounted by variation in temperature as high temperature in 15th December sowing and low temperature in 15th October sowing causes more accumulation of compatible solutes (Proline, glycine betaines and TSC) that might be responsible for stabilization and maintenance the structure of these enzymes that ultimately resulted into increased specific activities of enzymes in 15th December and 15th October sowing than 15th November sowing. Similar results also reported earlier through various studies in different plants by (Gill and Tuteja, 2010 [8]; in wheat and Kaushal *et al.*, 2011 [9]; Hasanuzzaman *et al.*, 2012 [10]; Kumar *et al.*, 2012 [3] in chickpea).

In fig. 2.11 data showed that among three dates of sowings, maximum accumulations of Ascorbate content ($0.91 \mu\text{ mol g}^{-1}\text{ DW}$) in leaf were recorded in crop sown on December 15th followed by 15th October ($0.91 \mu\text{ mol g}^{-1}\text{ DW}$) and minimum ($0.56 \mu\text{ mol g}^{-1}\text{ DW}$) were observed in November 15th sowing at 50% flowering stages. This might be accounted by variation in temperature as high temperature in 15th December sowing and low temperature in 15th October sowing causes more accumulation of compatible solutes (Proline, glycine betaines and TSC) that might be responsible for stabilization and maintenance the structure of these enzymes that ultimately resulted into increased specific activities of enzymes in 15th December and 15th October sowing than 15th November sowing. Similar results also reported earlier through various studies in different plants by (Gill and Tuteja, 2010 [8]; in wheat and Kaushal *et al.*, 2011 [9]; Hasanuzzaman *et al.*, 2012 [10]; Kumar *et al.*, 2012 [3] in chickpea).

In fig. 2.12 data showed that among three dates of sowings, maximum accumulations of Glutathione content ($1.24 \mu\text{ mol g}^{-1}\text{ DW}$) in leaf

were recorded in crop sown on December 15th followed by 15th October (0.91 $\mu\text{mol g}^{-1}\text{DW}$) and minimum (0.75 $\mu\text{mol g}^{-1}\text{DW}$) were observed in November 15th sowing at 50% flowering stages. This might be accounted by variation in temperature as high temperature in 15th December sowing and low temperature in 15th October sowing causes more accumulation of compatible solutes (Proline, glycine betaines and TSC) that might be responsible for stabilization and maintenance the structure of these enzymes that ultimately resulted into increased specific activities of enzymes in 15th December and 15th October sowing than 15th November sowing. Similar results also reported earlier through various studies in different plants by (Gill and Tuteja, 2010 [8]; in wheat and Kaushal *et al.*, 2011 [9]; Hasanuzzaman *et al.*, 2012 [10]; Kumar *et al.*, 2012 [3] in chickpea).

Seed yield (kg/ha)

Seed yield also differed significantly in all three dates of sowing. Maximum seed yield was observed in genotype H13-01 (2325 kg/ha) whereas minimum seed yield was observed in genotype H14-04 (1593 kg/ha) when considered irrespective of sowing dates (fig. 2.13). The minimum seed yield (1889 kg/ha) was observed in plants sown on 15th December and maximum seed yield (2113 kg/ha) was observed on 15th October sown crop when considered irrespective of genotypes. Among interactions, maximum seed yield was observed (2437 kg/ha) in genotype H13-01 when sown on 15th October whereas minimum seed yield (1656 kg/ha) was observed in genotype H14-04 when sown on 15th December. This might be due to comparatively favorable climatic conditions such as temperature, photoperiod, sunshine hours and higher values of leaf area, total dry matter, photo-

synthetic rate and chlorophyll content in 15th October sowing than 15th November and 15th December sowing. Lower seed yield during late sowing (15th December) might be due to shorter grain filling period as a result of high temperature at grain filling stages. Similar result also reported earlier in the literature (Kabir *et al.*, 2009 [38]; Krishnamurthy *et al.*, 2011 [25]; Mrudula *et al.*, 2013 [39]; Pawar, 2015 [40]; Husnain *et al.*, 2015 [41]; Nawab *et al.*, 2015; Patil *et al.*, 2017 [42]; Ali *et al.*, 2018 [43]; Bhaskar *et al.*, 2018) [44] in chickpea at different sowing dates.

Conclusion

Based upon antioxidants defence system and their contribution to seed yield, chickpea genotypes H12-64 and H13-01 were found to be promising in all the sowing dates and can be used in further breeding programme of chickpea for early (cold tolerance) and late (heat tolerance) sown conditions. It is concluded that these genotypes in future may prove better for late sown conditions (heat tolerance) and can be used in hybridization programme of chickpea. Maximum antioxidants and seed yield were recorded between 15th October and 15th November sowing, however, further delay in sowing to 15th December, significant reduction in antioxidants as well as seed yield were recorded.

Acknowledgement

The authors are grateful to the department of Botany and Plant Physiology for providing all the necessary facilities to carry out the research work.

Table 1.1. Changes in pollen viability (%) of chickpea genotypes at 50 % flowering under different sowing dates

Genotypes	Pollen viability (%)			
	Sowing dates			
	15 th October	15 th November	15 th December	Mean
H12-64	70.89	72.97	69.28	71.05
H13-01	71.23	74.43	70.89	72.19
H13-02	70.28	72.53	66.54	69.79
H14-01	69.24	71.53	64.17	68.31
H14-04	61.91	62.53	56.98	60.47
HC 1	64.94	66.50	62.68	64.71
HC 3	66.45	68.70	64.17	66.44
HC 5	65.50	67.50	63.98	65.66
ICCV 88503	66.27	67.73	58.98	64.33
ICCV 92944	64.68	65.57	63.39	64.54
Mean	67.14	69.00	64.11	

C.D.at 5% Dates of sowing (D) = 1.02 ; Genotypes (G) = 2.09 ; D x G = 3.62

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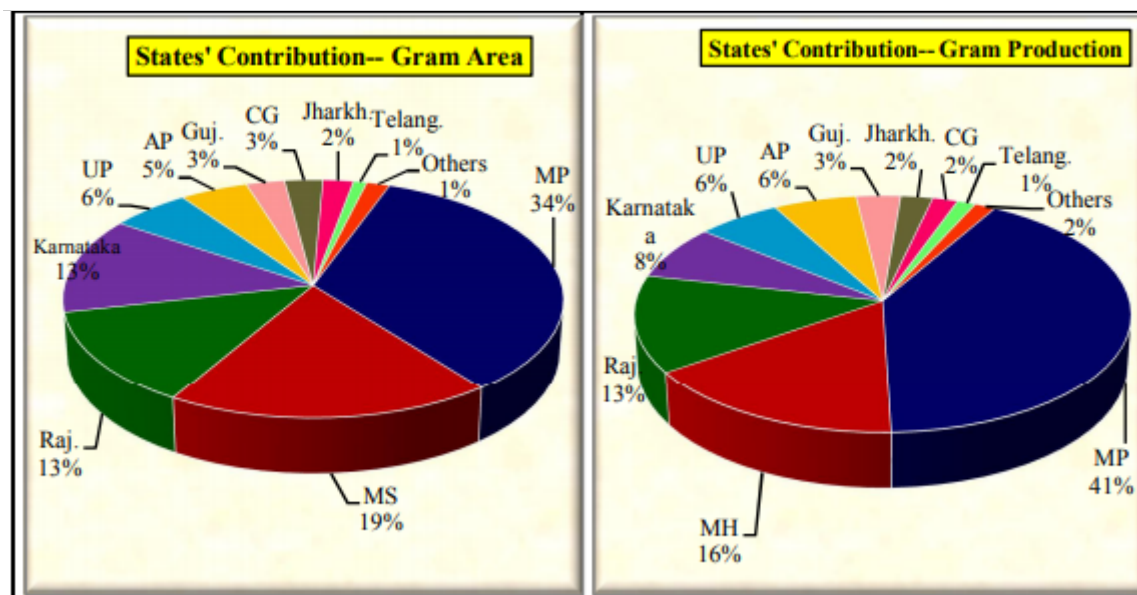


Figure 1.1. State wise contribution of area and production of chickpea during 2017–18 in India. (*Source - Anonymous, 2017-18) [45]

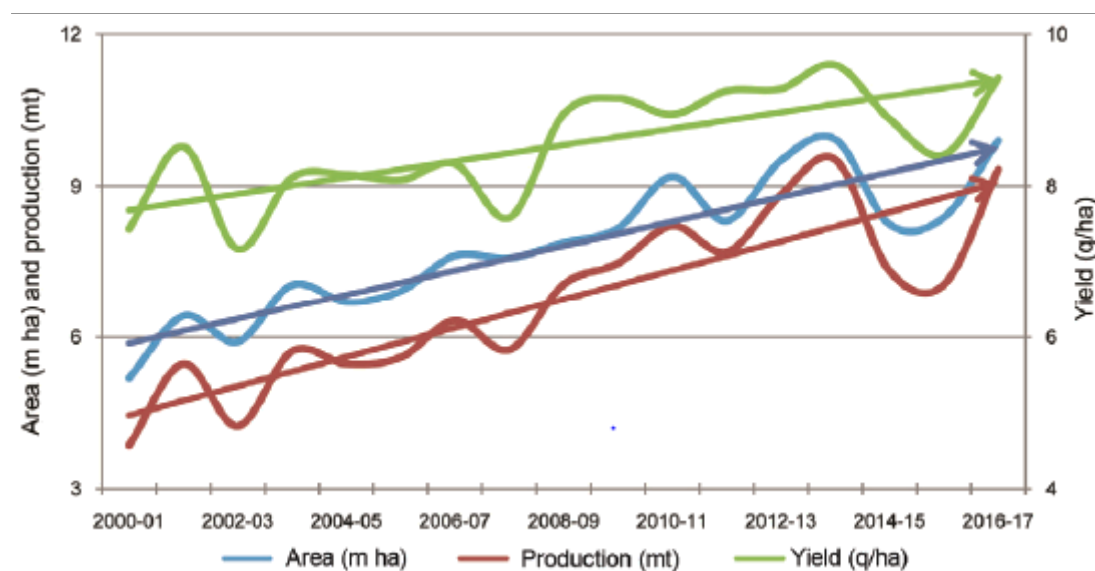


Figure 1.2. Area, production and yield of chickpea during 2000–01 to 2016–17 in India. (*Source - Anonymous, 2017-18) [45]

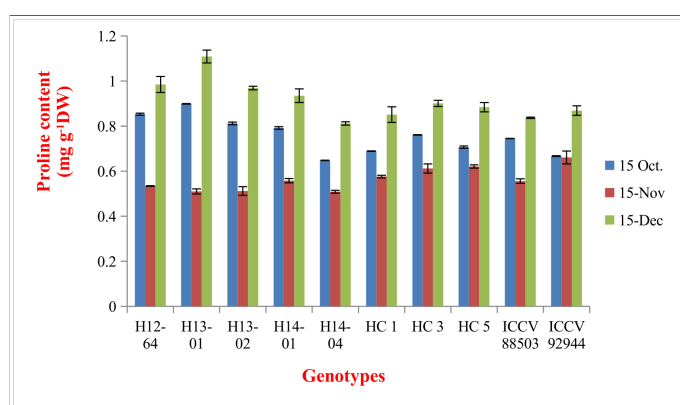


Fig: 2.1. Changes in proline content (mg g⁻¹ DW) of chickpea genotypes at 50 % flowering under different sowing dates [Vertical bars indicate $\pm$ SE for each treatment]

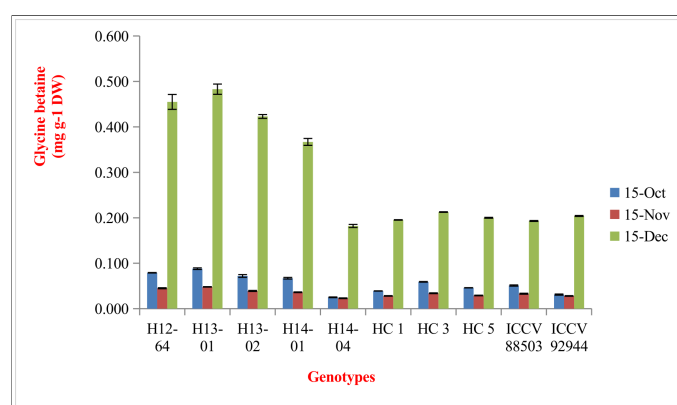


Fig: 2.2. Changes in glycine betaine content (mg g⁻¹ DW) of chickpea genotypes at 50 % flowering under different sowing dates [Vertical bars indicate $\pm$ SE for each treatment]

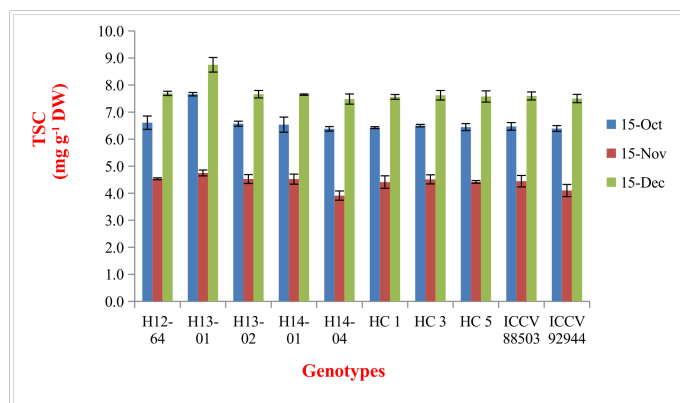


Fig: 2.3. Changes in total soluble carbohydrates (TSC) content (mg g⁻¹ DW) of chickpea genotypes at 50 % flowering under different sowing dates [Vertical bars indicate ± SE for each treatment]

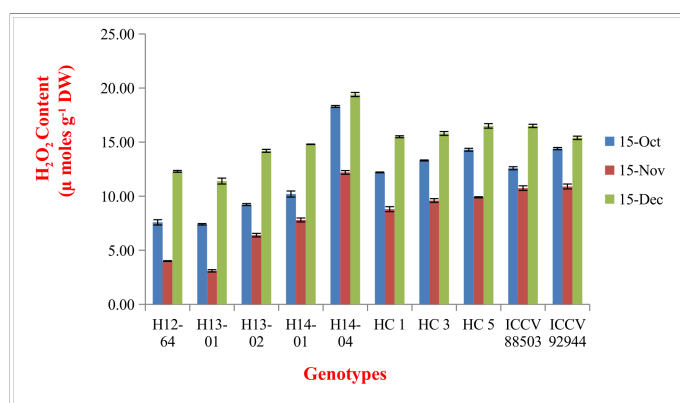


Fig: 2.4 Changes in H₂O₂ content (μ moles g⁻¹ DW) of chickpea genotypes at 50 % flowering under different sowing dates [Vertical bars indicate ± SE for each treatment]

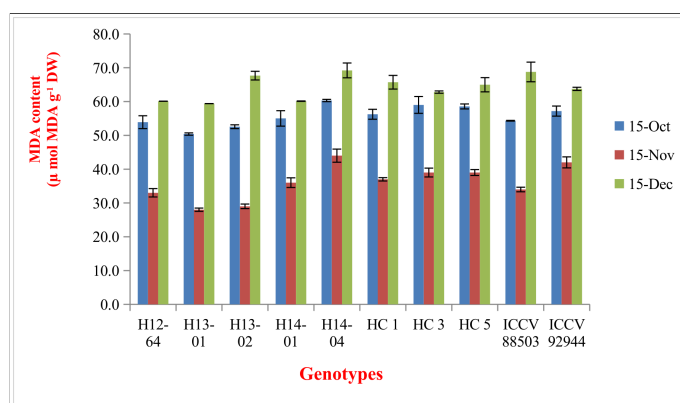


Fig: 2.5 Changes in malondialdehyde (MDA) content (μ moles MDA g⁻¹ DW) of chickpea genotypes at 50 % flowering under different sowing dates [Vertical bars indicate ± SE for each treatment]

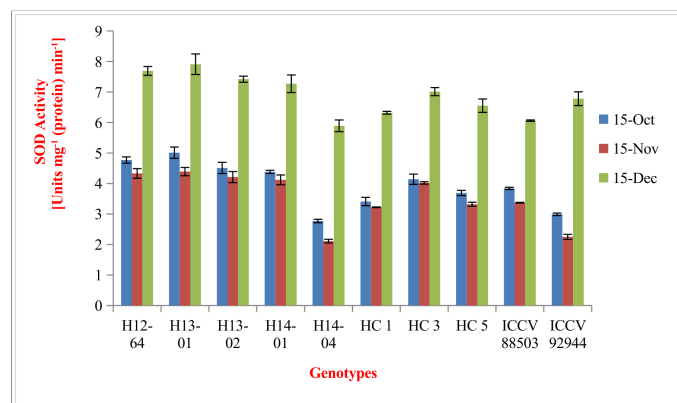


Fig: 2.6 Changes in superoxide dismutase (SOD) activity {Units mg⁻¹ (protein) min⁻¹} of chickpea genotypes at 50 % flowering under different sowing dates [Vertical bars indicate ± SE for each treatment]

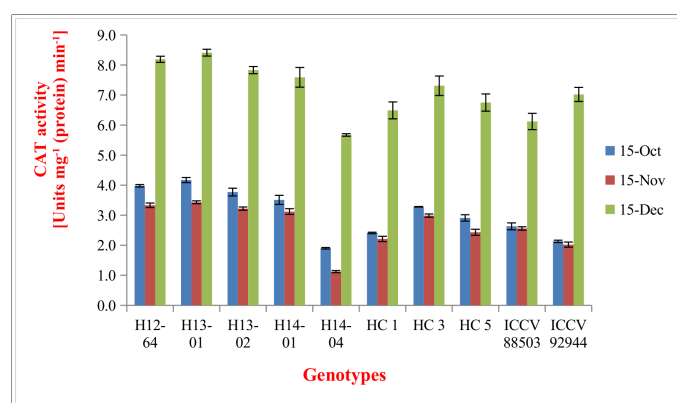


Fig: 2.7 Changes in catalase (CAT) activity {Units mg⁻¹ (protein) min⁻¹} of chickpea genotypes at 50 % flowering under different sowing dates [Vertical bars indicate ± SE for each treatment]

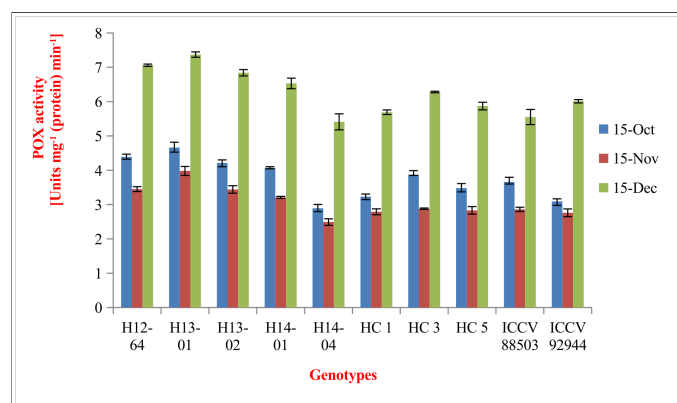


Fig: 2.8 Changes in peroxidase (POX) activity {Units mg⁻¹ (protein) min⁻¹} of chickpea genotypes at 50 % flowering under different sowing dates [Vertical bars indicate ± SE for each treatment]

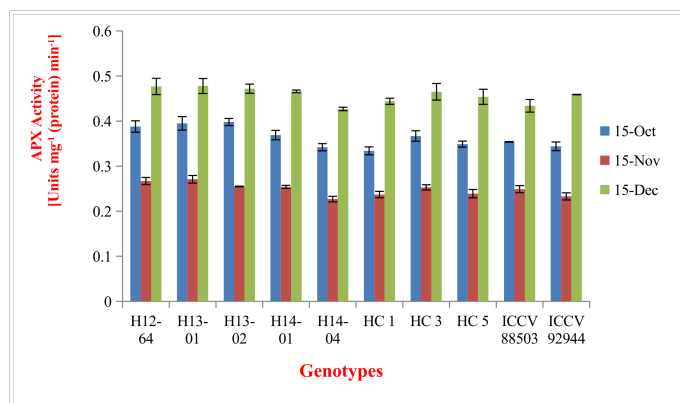


Fig: 2.9 Changes in ascorbate peroxidase (APX) activity {Units mg⁻¹ (protein) min⁻¹} of chickpea genotypes at 50 % flowering under different sowing dates [Vertical bars indicate ± SE for each treatment]

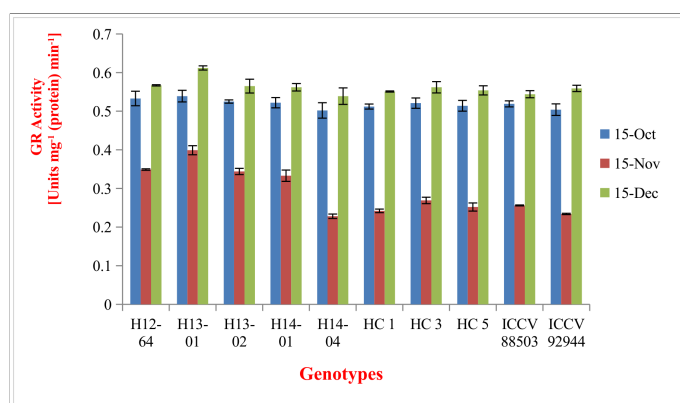


Fig: 2.10 Changes in glutathione reductase (GR) activity {Units mg⁻¹ (protein) min⁻¹} of chickpea genotypes at 50 % flowering under different sowing dates [Vertical bars indicate ± SE for each treatment]

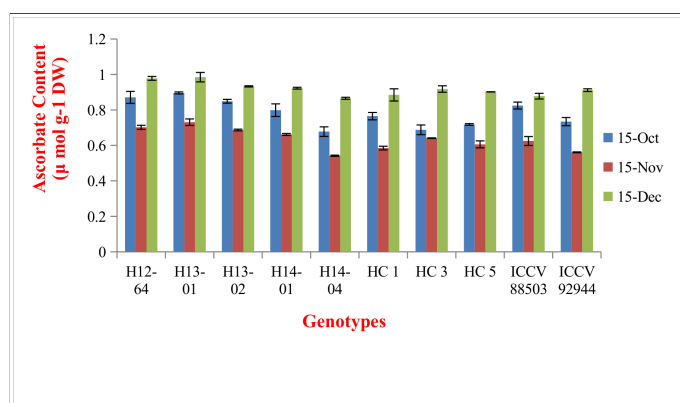


Fig: 2.11 Changes in ascorbate content (μ mol g⁻¹ DW) of chickpea genotypes at 50 % flowering under different sowing dates [Vertical bars indicate ± SE for each treatment]

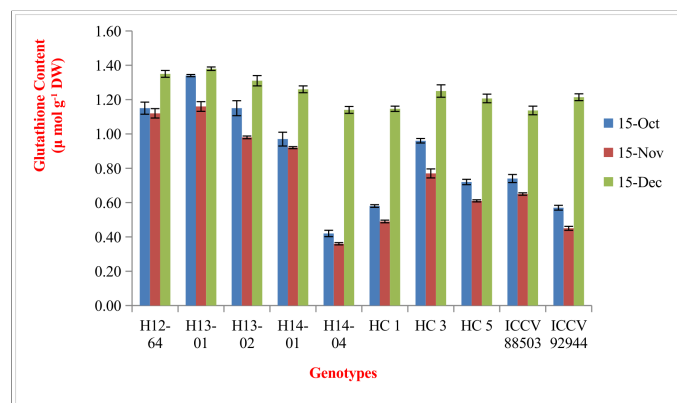


Fig: 2.12 Changes in glutathione content (μ mol g⁻¹ DW) of chickpea genotypes at 50% flowering under different sowing dates [Vertical bars indicate ± SE for each treatment]

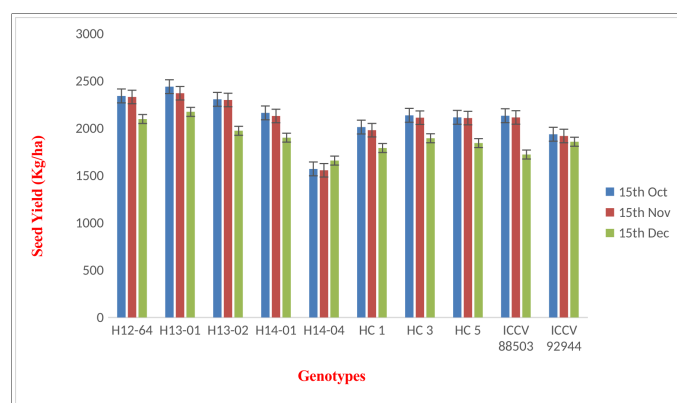


Fig: 2.13 Changes in seed yield (kg/ha) of chickpea genotypes at 50% flowering under different sowing dates [Vertical bars indicate ± SE for each treatment]

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