Studies on effect of terminal heat stress on seed quality and its mitigation in chickpea (*Cicer arietinum L.*)

Abstract

A field experiment was conducted to know the effect of terminal heat stress on seed yield and its mitigation in chickpea was carried out during 2021-22 at Seed Unit, University of Agricultural Sciences, Raichur. High temperature during sowing time from early to late and very late directly affect the vegetative and anthesis stages and it was overcome by spraying the plants with heat stress mitigating chemicals. The experiment was laid out with three dates of sowing and ten foliar spray each treatment was replicated twice in a split plot design. Spraying was done at two stages of crop growth *i.e*., at vegetative (35-40 DAS) and anthesis stage (50-60 DAS) in all dates of sowing. The interaction between dates of sowing and heat stress mitigating chemicals showed highest physiological and seed quality parameters *i.e.* chlorophyll stability index (D_3T_3) (73.06 and 74.63%), minimum cell membrane injury index (55.09 and 63.63 %), maximum proline content (D_3T_3) (6.79 and 9.37 μ mol g^{-1}), relative water content (83.79 and 75.10 %) at 60 DAS and at harvest, first count (90.50 %), final count (100.00 %), speed of germination (43.80), minimum time for radicle emergence (41.00 hrs), root length (17.32 cm), shoot length (10.12 cm), dry weight (27.10 mg), seedling vigour index-I (2643), seedling vigour index-II (2700).

Key words: chickpea, dates of sowing, heat stress, foliar spray

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2. INTRODUCTION

Chickpea (*Cicer arietinum* L.) is an annual legume and is the third most consumed legume crop, which is widely cultivated as a winter crop for its typically yellow-brown, pea like seeds in arid and semi-arid areas around the world. Chickpea (*Cicer arietinum* L.) belongs to genus Cicer, tribe cicerace, family sp. Fabaceae and subfamily Faboideae. Chickpea popularly known as gram, bengal gram, homes, chhola, garbanzo bean is one of the first seed legumes to be domesticated by humans in old world.

Heat stress is the increasing temperature over the optimum range of temperature during the growth and development of plant. Late planting of chickpea in India is very common due to the wide spread intensive cropping system which often delays the sowing of chickpea. As a result, portion of the maturity period of the crop is pushed forward and thus has to face higher temperature of the summer as well as hot spells, often occurring at the time of maturity.

Reproductive growth stage (flowering and podding) in chickpea is known to be very sensitive to changes in external environment and heat stress at this stage leads to reduction in seed yield. Drastic reductions in chickpea seed yields were observed when plants at flowering and pod development stages were exposed to high (35°C) temperatures (Summerfield *et al.*, 1984). Heat stress adversely affects pollen viability, fertilization, seed development, plant photosynthesis, growth, development, reproduction and metabolism. Therefore, the seriousness of high temperature stress depends on its timing, duration and intensity on crops.

Foliar spray is a technique of feeding nutrients to plant by applying liquid chemicals directly to crop canopy. If used widely, can more efficient, economical, environment friendly, target oriented when used to supplement soil fertilization. Now-a-days, foliar spray is widely adopted strategy in modern crop management where to ensure higher or optimum crop performance by enhancing crop growth. Foliar application overcome soil fertilization limitations, soil unsuitable for fertilizer precipitation, antagonism between certain nutrients, heterogenic soil unsuitable for low dosages and fixation.

Chickpea is grown during *rabi* season under reducing soil moisture conditions without any irrigation. As a result, there was water deficit for crop at critical stages which affects nutrient uptake ultimately causing yield reduction. To increase the yield during heat stress conditions, we have to take into consideration not only the normalization of plant water regime, but also the normalization of plant feeding and elimination of created deficiencies of some elements. Hence, various foliar spray chemicals used for heat stress mitigation can be helpful for achieving better yield from heat affected plants. Various studies have reported that foliar application of plants improves tolerance to heat stress as compared to non-sprayed plants. Through the present investigation, the conditions of cool winter followed by terminal heat stress which is prevalent in the northern dry zone of Karnataka is trying to mitigate by using heat stress mitigating chemicals at different sowing dates.

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2. METHODOLOGY

A field experiment was conducted at the seed production block, Seed Unit, Monitoring Agricultural Resources, University of Agricultural Sciences, Raichur, Karnataka. The crop was sown at three different times to achieve normal (October 1st fortnight), late (November 1st fortnight) and very late sowing (December 1st fortnight) conditions (Plate 1) in split-plot design with ten foliar spray treatments viz., control, salicylic acid (800 ppm and 400 ppm), ascorbic acid (10 ppm), KCl (1%), thiourea (400 ppm), cycocel (1000 ppm), KNO3 (0.3%), chickpea magic (8g/1), gibberellic acid (100ppm) each treatments was replicated twice in a split plot design during *rabi* season 2020-22. Spraying was done at two stages of crop growth i.e., at vegetative (35-40 DAS) and anthesis stage (50-60 DAS) in all dates of sowing.

2.1 Physiological parameters

2.1.1 Chlorophyll stability index (%)

Green plants pigments are thermo-sensitive and degradation occurs when they are subjected to higher temperature. This method is based on pigment changes induced by heating. Chlorophyll stability is the function of temperature and this property of chlorophyll stability was found to have good correlation with drought resistance. Representative leaf sample was placed in two clean tubes with 50 ml of distilled water. One tube was then subjected to heat on water bath at 65 ºC ± 1ºC for exactly 30 minutes. The chlorophyll in both the samples was extracted by placing the sample in 7 ml of DMSO at 65 ºC for 30 minutes. The supernatant was decanted and the tissue will be discarded, then volume was made to 10 ml by DMSO. Finally, the absorbance of the extract was read at 645, 652 and 663 nm using DMSO as blank (Hiscox and Isrealstam, 1979).

2.1.2 Cell membrane injury index (%)

The membrane injury index (MII) will be determined according to the method of Premchandra *et al*. (1990). Shoot portion (0.1 g) different treatments and control were horoughly washed in running tap water and double distilled water and thereafter placed 10 ml of double distilled water at 40 °C for 30 minutes. After the end of this period their electrical conductivity was recorded by EC meter (C_1). Subsequently the same samples were placed on boiling water bath (100 °C) for 10 min and their electrical conductivity is recorded as above (C_2).

2.1.3 Proline content (μ mol g^{-1} fr.wt.)

Leaf sample (0.5 g) was homogenized in 5.0 ml of sulphosalicylic acid (3%) using mortar and pestle. The homogenate is filtered through whatman No. 1 filter paper and filtrate was collected, which was used for the estimation of protein content. 2.0 ml of extract was taken in test tube and to it 2.0 ml of glacial acetic acid was added. The reaction mixture was heated in boiling water bath at 100 ºC for 30 min brick red color developed after cooling the reaction mixture, 6.0 ml of toluene was added and then transferred to a separating funnel (Plate 2). After through mixing the chromophore containing toluene through mixing the chromophore containing toluene was separated and its absorbance read at 520 nm in spectrophotometer against toluene blank.

2.1.4 Relative water content (%)

Plant leaves generally have lower (more negative) water potential than pure water (0.0), Hence, they osmotically absorb water and become turgid. A measure of this property is relative water content (RWC) which expresses the leaf water content (%) of the turgid leaf water content (Plate 5).

The relative water content was estimated based on the method by Barrs and Weatherley (1962). The leaf discs were taken from 3rd fully maturated leaf from top of the plant tip and were weighed to indicated as fresh weight (FW). Immediately after weighing, the leaf discs were transferred to petri dishes containing water. After 24 hours, leaf material was surface blotted and were weighed to indicated as turgid weigh (Plate 3). The leaf discs were then oven dried at 80 ºC up to 48 hrs and their dry weight was recorded. By using all these parameters, relative water content was calculated.

2.2 Seed quality parameters

2.2.1 First count

The seed germination test was conducted in four replicates of 100 seeds each by following between paper method and the rolled towels was incubated in the walk-in seed germination chamber maintained at 25 ± 2 ºC temperature and 90 ± 5 per cent relative humidity. The number of normal seedlings from each replication will be counted at the end of the 5 days and the mean germination percentage was calculated.

$$\text{Germination (\%)} = \frac{\text{Number of normal seedlings}}{\text{Total number of seeds}} \times 100$$

2.2.2 Final count

The seed germination test was conducted in four replicates of 100 seeds each by following between paper method and the rolled towels was incubated in the walk-in seed germination chamber maintained at 25 ± 2 ºC temperature and 90 ± 5 percent relative humidity. The number of normal seedlings from each replication was counted at the end of the 8 days and the mean germination percentage was calculated.

$$\text{Germination (\%)} = \frac{\text{Number of normal seedlings}}{\text{Total number of seeds}} \times 100$$

2.2.3 Speed of germination

The speed of germination was calculated by using the formula suggested by Maguire (1962).

$$\text{Speed of germination} = G_1/D_1 + G_2/D_2 + \ldots\ldots + Gn/Dn$$

Where,

G_1, G_2……………. Gn are the number of seeds germinated on D_1, D_2………...Dn day.

2.2.4 Time for radical emergence

From the germination test, 10 seeds were randomly selected from each treatment and replication and looked into it for every 4 hours to check the emergence of radical upto 2 mm in length. The time taken for emergnce is recorded and the mean time was calculated and expressed in hours.

2.2.5 Root length (cm)

From the germination test, ten normal seedlings were selected randomly from each treatment on 8th day. The root length was measured from the tip of primary root to base of hypocotyls and mean root length was expressed in centimeters.

2.2.6 Shoot length (cm)

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From the germination test, the ten random seedlings were used for measuring shoot length. The shoot length was measured from the base of primary leaf to the base of hypocotyls and the mean shoot length was expressed in centimeter.

2.2.7 Dry weight (mg)

From the germination test the same ten seedlings used for measuring the root and shoot length along with another five seedlings was kept in a butter paper packed and dried in hot air oven maintained at 70 ºC for 24 hours. Then the seedlings were cooled in a desiccator for 30 minutes and the weight of dried seedling was recorded using an electronic balance and was expressed in milligram.

2.2.8 Seedling vigour index - I

The seedling vigour index-I was computed using the formula as suggested by Abdul-Baki and Anderson (1973) as follows

$$\text{Seedling vigour index-I} = \text{Germination (\%)} \times \text{Mean seedling length (cm)}$$

2.2.9 Seedling vigour index - II

The seedling vigour index-II was computed by multiplying the germination (%) with seedlings dry weight (g) as follows.

2.3 Statistical analysis and interpretation of data

In order evaluate comparative performance of various treatments, the data was analyzed by the technique of analysis of variance given by Fischer (1950). The collected research data were analyzed statistically by the method of Panse and Sukhatme (1967) wherever the results were significant, the critical difference (CD) was calculated at 1 percent level of significance for laboratory observation ($P < 0.05$).

3 RESULTS AND DISCUSSION

3.1 Physiological parameters

Chlorophyll is one of the major components of the chloroplast and is found to be positively correlated with the photosynthetic rate. High temperature has been found to be associated with chlorophyll content and the ability to stay green. The Chlorophyll stability

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index showed non-significant results due to the interaction effect of date of sowing and heat stress mitigating chemicals was shown in Table 1. Among the interactions, normal date of sowing and foliar spray of salicylic acid @ 400 ppm (D_1T_3) exhibited lowest chlorophyll stability index (67.76 and 71.06 %) at 60 DAS and at harvest, and it was followed by normal dates of sowing and foliar spray of gibberellic acid @ 100 ppm (D_1T_{10}) (65.74 and 69.36 %) as compared to all other treatments. However, plants sprayed with salicylic acid @ 400 ppm under very late sowing recorded the maximum chlorophyll stability index (D_3T_3) (73.06 and 74.63%) at 60 DAS and at harvest. Whereas plants without any spray under very late sowing recorded minimum chlorophyll stability index (D_3T_1) (65.96 and 67.63 %) at 60 DAS and at harvest. This increase in chlorophyll stability index at 60 days after sowing and at harvest was observed in salicylic acid sprayed plants under very late sowing condition compared to control might be due to increased activity of chlorophyll which deal with the heat stress leads to increase in chlorophyll in soybean (Ghassemi-Golezani *et al.* 2018).

The membrane injury index is another physiological index that has been widely used to evaluate heat tolerance. Interaction between date of sowing and heat stress mitigating chemicals, the cell membrane injury index showed non-significant results was shown in Table 1. Normal date of sowing and plants sprayed with salicylic acid @ 400 ppm (D_1T_3) exhibited the minimum cell membrane injury index (55.09 and 63.63 %) at 60 DAS and at harvest and it was followed by normal dates of sowing and foliar spray of gibberellic acid @ 100 ppm (D_1T_{10}) (56.17 and 64.05 %) as compared to all other treatments. However, very late sowing and plants sprayed with salicylic acid @ 400 ppm (D_3T_3) (57.63 and 66.12) exhibited the minimum cell membrane injury index when compared to control. Whereas very late sowing without any spray recorded the maximum cell membrane injury index (D_3T_1) (72.03 and 73.15 %) at 60 DAS and at harvest. This decrease in cell membrane injury index at 60 days after sowing and at harvest was observed in salicylic acid sprayed plants under very late sowing condition compared to control might be due to exogenously applied salicylic acid significantly reduced the ion leakage and lipid peroxidation that acts to deal with heat stress leading to higher chlorophyll content in chickpea (Khetrapal *et al.* 2009).

Further, due to the interaction effect between date of sowing and heat stress mitigating chemicals, proline content showed non-significant results was shown in Table 2 (Plate 4). Interaction between normal date of sowing and foliar spray of salicylic acid @ 400 ppm (D_1T_3) exhibited minimum proline content (4.68 and 8.99 μ mol g^{-1}) at 60 DAS and at

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harvest, and it was followed by normal dates of sowing and foliar spray of gibberellic acid @ 100 ppm (D_1T_{10}) (4.54 and 8.75 μ mol g^{-1}) as compared to control. However, plants sprayed with salicylic acid @ 400 ppm under very late sowing condition recorded the maximum proline content (D_3T_3) (6.79 and 9.37 μ mol g^{-1}) at 60 DAS and at harvest. Whereas plants sprayed with salicylic acid @ 400 ppm (D_1T_1) under normal sowing conditions recorded lowest proline content (5.63 and 8.79 μ mol g^{-1}) at 60 DAS and at harvest. In this present study, the results indicated that, 17.0 and 6.1 per cent increase in proline content at 60 days after sowing and at harvest was observed in salicylic acid sprayed plants under very late sowing condition compared to control might be due to salicylic acid respond to heat stress by accumulating certain specific metabolites such as amino acids, proteins and proline under stress, the increase in proline content had beneficial in enhancing plant resistance to stress thereby developing adaptations in plants to survive under environmental stress in black gram (Baroowa and Gogoi 2012).

Relative water content showed non-significant results due to the interaction of date of sowing and heat stress mitigating chemicals was shown in Table 2. The interactions between normal date of sowing and plants sprayed with salicylic acid @ 400 ppm (D_1T_3) exhibited maximum relative water content (83.79 and 75.10 %) at 60 DAS and at harvest and it was followed by normal dates of sowing and foliar spray of gibberellic acid @ 100 ppm (D_1T_{10}) (82.96 and 74.31 %) as compared to all other treatments. Whereas minimum relative water content was recorded under very late sowing without any spray (D_3T_1) (61.21 and 59.93 %). In the present study, salicylic acid sprayed plants showed 12.0 and 7.2 per cent increase in relative water content at 60 days after sowing and at harvest under very late sowing conditions compared with control, this might be due to the fact that salicylic acid respond to heat stress from osmoregulation by increasing the production of osmolytes, as ions or sugars are often accumulated in plants under heat stress conditions in chickpea (Gunes *et al*. 2008).

3.2 Seed quality parameters

First count showed non-significant results due to interaction between date of sowing and heat stress mitigating chemicals was shown in (Table 3). Among the interactions, normal date of sowing and foliar spray of salicylic acid @ 400 ppm (D_1T_3) exhibited maximum first count (90.50 %) and it was followed by normal dates of sowing and foliar spray of gibberellic acid @ 100 ppm (D_1T_{10}) (90.00 %) as compared to all other treatments. Whereas very late sowing without any spray recorded the minimum first count (control) (D_3T_1) (86.50 %).

Interaction between date of sowing and heat stress mitigating chemicals, final count yielded non-significant results was shown in Table 3 (Plate 5). Interaction between normal date of sowing and foliar spray of salicylic acid @ 400 ppm (D_1T_3) exhibited maximum final count (100.00 %) and it was followed by normal dates of sowing and foliar spray of gibberellic acid @ 100 ppm (D_1T_{10}) (99.00 %) as compared to all other treatments. Whereas very late sowing without any spray recorded the minimum final count (D_3T_1) (97.50 %).

The non-significant variation was recorded for speed of germination due to interaction between sowing date and heat stress mitigating chemicals was shown in Table 3 (Plate 5). Among the interaction, normal date of sowing and foliar spray of salicylic acid @ 400 ppm (D_1T_3) exhibited the maximum speed of germination (43.80) and was followed by normal dates of sowing and foliar spray of gibberellic acid @ 100 ppm (D_1T_{10}) (42.50) as compared to all other treatments. Whereas minimum speed of germination was recorded under late sowing without any spray (D_3T_1) (36.70). The increase in germination under normal sowing conditions compared to control might be due to plants sprayed with salicylic acid that induced genes encoding heat resistance on seed germination by enhancing the physiological activity and translocation of food reserves necessary for growth and increase in these traits may also be attributed to the role of salicylic acid in increasing oxygen and nutrient uptake and the activity of enzymes in seeds as reported by (Alamri *et al*., 2018) in wheat.

Interaction among the dates of sowing and heat stress mitigating chemicals showed non-siginificant results for time for radicle emergence was shown in Table 3. Among the interactions, the normal date of sowing and foliar spray of salicylic acid @ 400 ppm (D_1T_3) exhibited the minimum time for radicle emergence (41.00 hrs) and was followed by the normal dates of sowing and foliar spray of gibberellic acid @ 100 ppm (D_1T_{10}) (41.50 hrs) as compared to all other treatments. whereas very late sowing without any spray recorded the maximum time for radicle emergence (D_3T_1) (46.00 hrs). This quicker radicle emergence might be due to application of salicylic acid increases oxygen and nutrient uptake and the activity of enzymes in seeds in pulses (Mavi *et al*. 2010).

Root length showed non-significant results due to the interaction between date of sowing and heat stress mitigating chemicals was shown in Table 4 (Plate 6). Interaction

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between normal date of sowing and foliar spray of gibberellic acid @ 100ppm (D_1T_{10}) exhibited the maximum root length (17.32 cm) and it was followed by normal dates of sowing and foliar spray of salicylic acid @ 400 ppm (D_1T_3) (17.01 cm) as compared to all other treatments. Whereas very late sowing without any spray recorded the minimum seedling root length (D_3T_1) (15.91 cm). The two spray of gibberellic acid was given at vegetative and anthesis stage on chickpea showed the favourable effect on above parameter and it was also observed that, gibberellic acid act as a signal molecule and involved in several physiological processes controlling the seed germination that promotes stem cell elongation and root length under heat stress conditions in chickpea (Roychowdry *et al*. 2012).

Shoot length showed non-significant results due interaction effect between date of sowing and heat stress mitigating chemicals was shown in Table 4 (Plate 6). Among the interactions, normal date of sowing and foliar spray of gibberellic acid @ 100ppm (D_1T_{10}) exhibited the maximum shoot length (10.12 cm) and it was followed by normal dates of sowing and foliar spray of salicylic acid @ 400 ppm (D_1T_3) (9.13 cm) as compared to all other treatments. Whereas very late sowing without any spray recorded the minimum shoot length (D_3T_1) (7.82 cm). The experiment findings revealed that shoot length registered significantly superior in the treatment with plants sprayed with gibberellic acid was attributed to decreases the stress by increasing sucrose transport to shoots from cotyledons, which had been reduced under stress conditions and also by increasing invertase activity in shoots in chickpea (Mazid 2014).

Dry weight showed non-significant results due to interaction effect of date of sowing and heat stress mitigating chemicals was shown in Table 4. Interaction among the normal date of sowing and foliar spray of gibberellic acid @ 100ppm (D_1T_{10}) exhibited maximum dry weight (27.10 mg) and it was followed by normal dates of sowing and foliar spray of salicylic acid @ 400 ppm (D_1T_3) (27.00 mg) as compared to all other treatments. Whereas very late sowing without any spray recorded the minimum dry weight (D_3T_1) (22.10 mg). Moreover, plants sprayed with gibberellic acid responded better to above parameter under stress conditions may be due to enhanced the cell division and longer length in chickpea (Roychowdry *et al*. 2012).

Interaction between date of sowing and heat stress mitigating chemicals, the seedling vigour index-I showed non-significant results was shown in Table 4 (Plate 6). Among the interactions, normal date of sowing and foliar spray of gibberellic acid @ 100ppm (D_1T_{10})

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exhibited the maximum seedling vigour index-I (2716) and it was followed by normal dates of sowing and foliar spray of salicylic acid @ 400 ppm (D_1T_3) (2614) as compared to all other treatments. Whereas very late sowing without any spray recorded the minimum seedling vigour index-I (D_3T_1) (2313).

Seedling vigour index-II showed non-significant results due to ineraction between date of sowing and heat stress mitigating chemicals was shown in Table 4 (Plate 6). Interaction between normal date of sowing and foliar spray of salicylic acid @ 400 ppm (D_1T_3) exhibited maximum seedling vigour index-II (2700) and it was followed by normal dates of sowing and foliar spray of gibberellic acid @ 100 ppm (D_1T_{10}) (2682) as compared to all other treatments. Whereas very late sowing without any spray recorded the minimum seedling vigour index-II (D_3T_1) (2154). This experiment revealed the minimum seedling vigour index under very late date of sowing might be due to rise in temperature might cause damage to membranes, cellular oxidizing ability and photochemical efficiency in shoots and also reduce the activity of proteins and enzymes under very late sowing conditions in chickpea (Kaur *et al.* 1998).

4 CONCLUSION

It can be concluded that, heat stress adversely affects the growth and seed yield of chickpea. Among the treatments, sowing done at October 15th and plants sprayed with salicylic acid @ 400 ppm twice at vegetative and anthesis stage was found to be better in obtaining significantly higher physiological and seed quality parameters in chickpea variety JG-11 under heat stress conditions.

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Table 1. Effect of dates of sowing and heat stress mitigating chemicals on chlorophyll stability index and cell membrane injury index in chickpea

Treatments	Chlorophyll stability index (%)								Cell membrane injury index (%)							
	At 60 DAS				At maturity				At 60 DAS				At maturity			
	D_1	D_2	D_3	Mean	D_1	D_2	D_3	Mean	D_1	D_2	D_3	Mean	D_1	D_2	D_3	Mean
T_1	60.04	62.17	65.96	62.72	63.96	65.89	67.63	65.83	70.31	71.69	72.03	71.34	71.38	72.91	73.15	72.48
T_2	63.63	66.02	68.92	66.19	67.45	69.17	70.52	69.05	60.76	62.09	63.54	62.13	65.61	67.1	67.83	66.85
T_3	67.76	70.01	73.06	70.28	71.06	73.18	74.63	72.96	55.09	56.47	57.63	56.4	63.63	64.61	66.12	64.79
T_4	62.67	65.41	67.43	65.17	66.79	68.13	70.01	68.31	62.61	64.5	65.37	64.16	66.09	67.49	68.23	67.27
T_5	61.02	64.23	66.23	63.83	65.26	67.32	69.2	67.26	66.81	70.27	71.73	69.6	69.71	71.28	72.53	71.17
T_6	62.93	65.28	67.56	65.26	66.74	68.24	70.54	68.51	64.01	65.49	66.82	65.44	66.81	68.42	69.96	68.4
T_7	62.09	66.09	68.32	65.5	65.93	67.62	69.93	67.83	65.28	66.59	67.91	66.59	67.81	69.3	70.81	69.31
T_8	63.06	66.53	68.26	65.95	67.06	68.45	71.02	68.84	62.21	63.64	65.07	63.64	65.09	67.01	67.12	66.41
T_9	64.19	67.08	69.03	66.77	67.93	69.01	70.34	69.09	58.83	60.13	61.57	60.18	64.67	66.09	67.16	65.97
T_{10}	65.74	67.86	69.93	67.84	69.36	70.93	72.73	71.01	56.17	57.47	58.86	57.5	64.05	65.45	66.72	65.41
Mean	63.31	66.07	68.47		67.15	68.79	70.66		62.21	63.83	65.05		66.49	67.97	68.96	
	D	**T**	**D x T**		**D**	**T**	**D x T**		**D**	**T**	**D x T**		**D**	**T**	**D x T**	
SEm±	0.006	1.032	1.789		0.001	1.08	1.871		0.008	1.038	1.799		0.001	1.088	1.885	
CD @ 5%	0.021	2.997	NS		0.01	3.134	NS		0.028	3.014	NS		0.01	3.159	NS	

NS: Non-significant

Sowing window

D_1 = Normal date of sowing

D_2 = Late date of sowing

D_3 = Very late date of sowing

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Mitigation treatments

T_1: Control
T_2: Salicylic acid (800 ppm)
T_3: Salicylic acid (400 ppm)
T_4: Ascorbic acid (10 ppm)
T_5: KCl (1%)
T_6: Thiourea (400 ppm)
T_7: Cycocel (1000 ppm)
T_8: KNO_3 (0.3%)
T_9: Chickpea magic (8g/l)
T_{10}: GA_3 (100ppm)

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Table 2. Effect of dates of sowing and heat stress mitigating chemicals on proline content and relative water content in chickpea

Treatments	Proline content (μ mol g^{-1})								Relative water content (%)							
	At 60 DAS				At maturity				At 60 DAS				At maturity			
	D_1	D_2	D_3	Mean	D_1	D_2	D_3	Mean	D_1	D_2	D_3	Mean	D_1	D_2	D_3	Mean
T_1	4.02	4.37	5.63	4.67	8.03	8.56	8.79	8.46	79.1	70.53	61.21	70.28	70.37	62.85	59.93	64.38
T_2	4.28	5.01	6.23	5.17	8.46	8.72	8.98	8.72	81.36	75.69	66.1	74.38	72.91	65.01	62.2	66.71
T_3	4.68	5.81	6.79	5.76	8.99	9.09	9.37	9.15	83.79	79.59	69.59	77.66	75.1	67.43	64.61	69.05
T_4	4.24	4.83	6.13	5.07	8.39	8.67	8.94	8.67	80.96	74.86	65.43	73.75	72.32	64.72	61.89	66.31
T_5	4.07	4.51	5.71	4.76	8.15	8.53	8.81	8.5	79.85	71.56	62.82	71.41	70.93	63.35	60.53	64.94
T_6	4.23	4.85	6.13	5.07	8.38	8.65	8.93	8.65	80.51	73.63	64.03	72.72	71.8	64.13	61.13	65.69
T_7	4.16	4.63	5.96	4.92	8.25	8.58	8.88	8.57	80.2	72.55	63.62	72.12	71.1	63.85	60.9	65.28
T_8	4.2	4.72	6.01	4.98	8.31	8.61	8.91	8.61	81.15	74.83	65.41	73.8	72.48	64.87	61.97	66.44
T_9	4.31	5.12	6.3	5.24	8.57	8.77	9.04	8.79	81.73	76.81	66.62	75.05	73.51	65.7	62.79	67.33
T_{10}	4.54	5.47	6.56	5.52	8.75	8.91	9.36	9.01	82.96	78.28	68.1	76.45	74.31	66.53	63.65	68.16
Mean	4.27	4.93	6.15		8.43	8.71	9		81.16	74.83	65.29		72.48	64.84	61.96	
	D	T	D x T		D	T	D x T		D	T	D x T		D	T	D x T	
SEm±	0.0006	0.0798	0.1382		0.0006	0.1364	0.2362		0.012	1.158	2.006		0.0014	1.043	1.807	
CD @ 5%	0.0025	0.2316	NS		0.0024	0.3957	NS		0.041	3.361	NS		0.058	3.028	NS	

NS: Non-significant

Sowing window

D_1 = Normal date of sowing

D_2 = Late date of sowing

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D_3 = Very late date of sowing

Mitigation treatments

T_1: Control
T_2: Salicylic acid (800 ppm)
T_3: Salicylic acid (400 ppm)
T_4: Ascorbic acid (10 ppm)
T_5: KCl (1%)
T_6: Thiourea (400 ppm)
T_7: Cycocel (1000 ppm)
T_8: KNO_3 (0.3%)
T_9: Chickpea magic (8g/l)
T_{10}: GA_3 (100ppm)

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Table 3. Effect of dates of sowing and heat stress mitigating chemicals on germination (first & final count), speed of germination and time for radical emergence in chickpea

Treatments	First count (%)				Final count (%)				Speed of germination				Time for radical emergence (hrs)			
	D_1	D_2	D_3	Mean	D_1	D_2	D_3	Mean	D_1	D_2	D_3	Mean	D_1	D_2	D_3	Mean
T_1	88	87.5	86.5	87.3	98.5	98.5	97.5	98.2	37.4	37.2	36.7	37.1	46	46	46	46
T_2	89.5	88.5	88.5	88.8	98.5	98.5	98	98.3	40.7	40.5	39.8	40.3	46	46	46	46
T_3	90.5	90.5	89.5	90.2	100	98.5	98	98.8	43.8	43.2	42.8	43.3	41	42	46	43
T_4	89.5	88.5	88	88.7	99	98.5	98	98.5	40.7	40.6	40.3	40.5	46	46	46	46
T_5	89.5	88.5	87	88.3	98.5	99	98	98.5	37.7	36.7	36.5	37	46	46	46	46
T_6	89.5	88.5	88	88.7	98.5	98	98	98.2	40.5	39.9	39.5	40	46	46	46	46
T_7	89.5	88.5	87.5	88.5	99	98	98.5	98.5	40.6	40.3	39.7	40.2	46	46	46	46
T_8	90	89.5	88.5	89.3	98.5	98	98.5	98.3	41.3	40.9	40.5	40.9	46	46	46	46
T_9	90	89	88.5	89.2	98.5	98	98.5	98.3	41.8	41.1	40.9	41.3	46	46	46	46
T_{10}	90	89	88.5	89.2	99	99	98.5	98.8	42.5	42.3	41.3	42	41.5	42	46	43.1
Mean	89.6	88.8	88.05		98.8	98.4	98.2		40.7	40.2	39.8		45	45.2	46	
	D	**T**	**D x T**		**D**	**T**	**D x T**		**D**	**T**	**D x T**		**D**	**T**	**D x T**	
SEm±	0.177	0.334	0.578		0.318	0.395	0.684		0.016	0.887	1.537		0.04	1.008	1.747	
CD @ 1%	0.565	0.985	NS		1.371	0.81	NS		0.05	2.821	NS		0.175	2.07	NS	

NS: Non-significant

Sowing window

D_1 = Normal date of sowing

D_2 = Late date of sowing

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D_3 = Very late date of sowing

Mitigation treatments

T_1: Control

T_2: Salicylic acid (800 ppm)

T_3: Salicylic acid (400 ppm)

T_4: Ascorbic acid (10 ppm)

T_5: KCl (1%)

T_6: Thiourea (400 ppm)

T_7: Cycocel (1000 ppm)

T_8: KNO_3 (0.3%)

T_9: Chickpea magic (8g/l)

T_{10}: GA_3 (100ppm)

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Table 4. Effect of dates of sowing and heat stress mitigating chemicals on in chickpea root length, shoot length, dry weight, seedling vigour-I & seedling vigour-II

Treatments	Root length (cm)				Shoot length (cm)				Dry weight (mg)				Seedling vigour index-I				Seedling vigour index-II			
	D_1	**D_2**	**D_3**	**Mean**	**D_1**	**D_2**	**D_3**	**Mean**	**D_1**	**D_2**	**D_3**	**Mean**	**D_1**	**D_2**	**D_3**	**Mean**	**D_1**	**D_2**	**D_3**	**Mean**
T_1	16.61	16.52	15.91	16.34	7.9	7.91	7.82	7.87	23.4	22.6	22.1	22.7	2414	2404	2313	2377	2304	2226	2154	2228
T_2	16.92	16.9	16.51	16.77	8.92	8.92	8.7	8.84	27	26.2	25	26.1	2545	2543	2470	2519	2659	2580	2450	2563
T_3	17.01	16.91	16.6	16.84	9.13	9.04	8.91	9.02	27	26.3	25.2	26.2	2614	2556	2499	2556	2700	2590	2469	2586
T_4	16.72	16.9	16.52	16.71	8.7	8.7	8.52	8.64	26	26	24.5	25.5	2516	2521	2453	2496	2574	2561	2401	2512
T_5	16.7	16.62	16	16.44	8.01	8.01	7.9	7.97	23.4	22.8	22.5	22.9	2433	2537	2342	2437	2304	2257	2205	2255
T_6	16.7	16.3	16.31	16.43	8.52	8.42	8.41	8.45	25	24	23	24	2484	2422	2423	2443	2462	2352	2254	2356
T_7	16.7	16.02	16.53	16.41	8.6	8.53	8.5	8.54	25.6	24.5	23.5	24.5	2504	2405	2465	2458	2534	2401	2314	2416
T_8	16.71	16.7	16.2	16.53	8.52	8.44	8.52	8.49	24.5	23.6	23	23.7	2485	2463	2434	2460	2413	2312	2265	2330
T_9	16.7	16.81	16.51	16.67	8.7	8.61	8.6	8.63	26	26	23.7	25.2	2501	2491	2473	2488	2561	2548	2334	2481
T_{10}	17.32	17.1	16.6	17	10.12	9.6	9.52	9.74	27.1	26.6	25.6	26.4	2716	2643	2572	2643	2682	2633	2521	2612
Mean	16.8	16.67	16.36		8.71	8.61	8.54		25.5	24.9	23.8		2521	2498	2444		2519	2446	2336	
	D	**T**	**D x T**		**D**	**T**	**D x T**		**D**	**T**	**D x T**		**D**	**T**	**D x T**		**D**	**T**	**D x T**	

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SEm±	0.002	0.263	0.455		0.002	0.133	0.23		0.002	0.384	0.6655		0.211	38.48	66.64		0.334	38.35	66.43	
CD @ 1%	0.007	NS	NS		0.006	0.386	NS		0.007	1.114	NS		1.088	111.6	NS		1.035	111.3	NS	

NS: Non-significant

Sowing window

D_1 = Normal date of sowing

D_2 = Late date of sowing

D_3 = Very late date of sowing

Mitigation treatments

T_1: Control

T_2: Salicylic acid (800 ppm)

T_3: Salicylic acid (400 ppm)

T_4: Ascorbic acid (10 ppm)

T_5: KCl (1%)

T_6: Thiourea (400 ppm)

T_7: Cycocel (1000 ppm)

T_8: KNO_3 (0.3%)

T_9: Chickpea magic (8g/l)

T_{10}: GA_3 (100ppm)

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Fig. 1. Effect of dates of sowing and heat stress mitigating chemicals on chlorophyll stability index in chickpea

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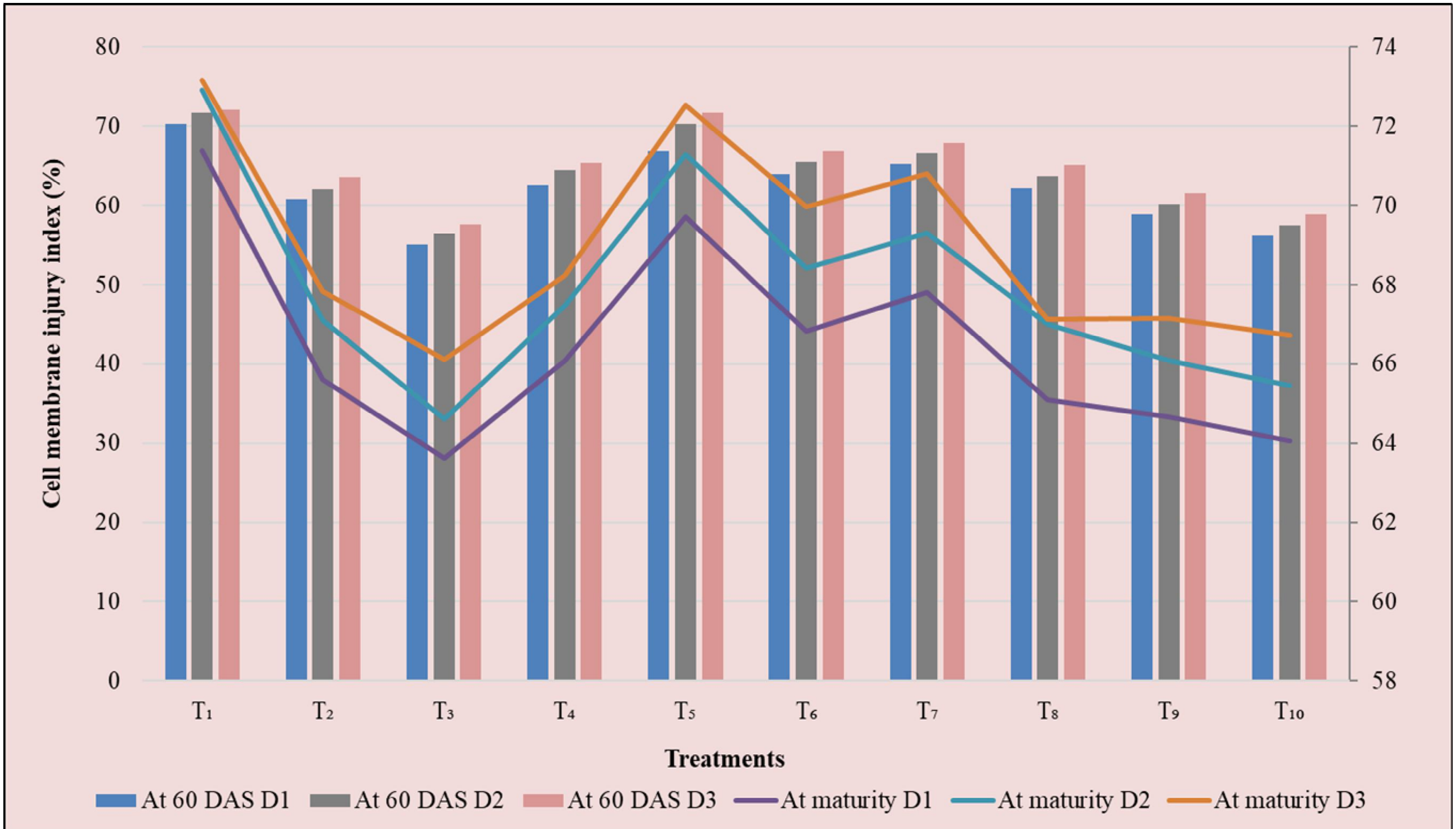

Fig. 2. Effect of dates of sowing and heat stress mitigating chemicals on cell membrane injury index

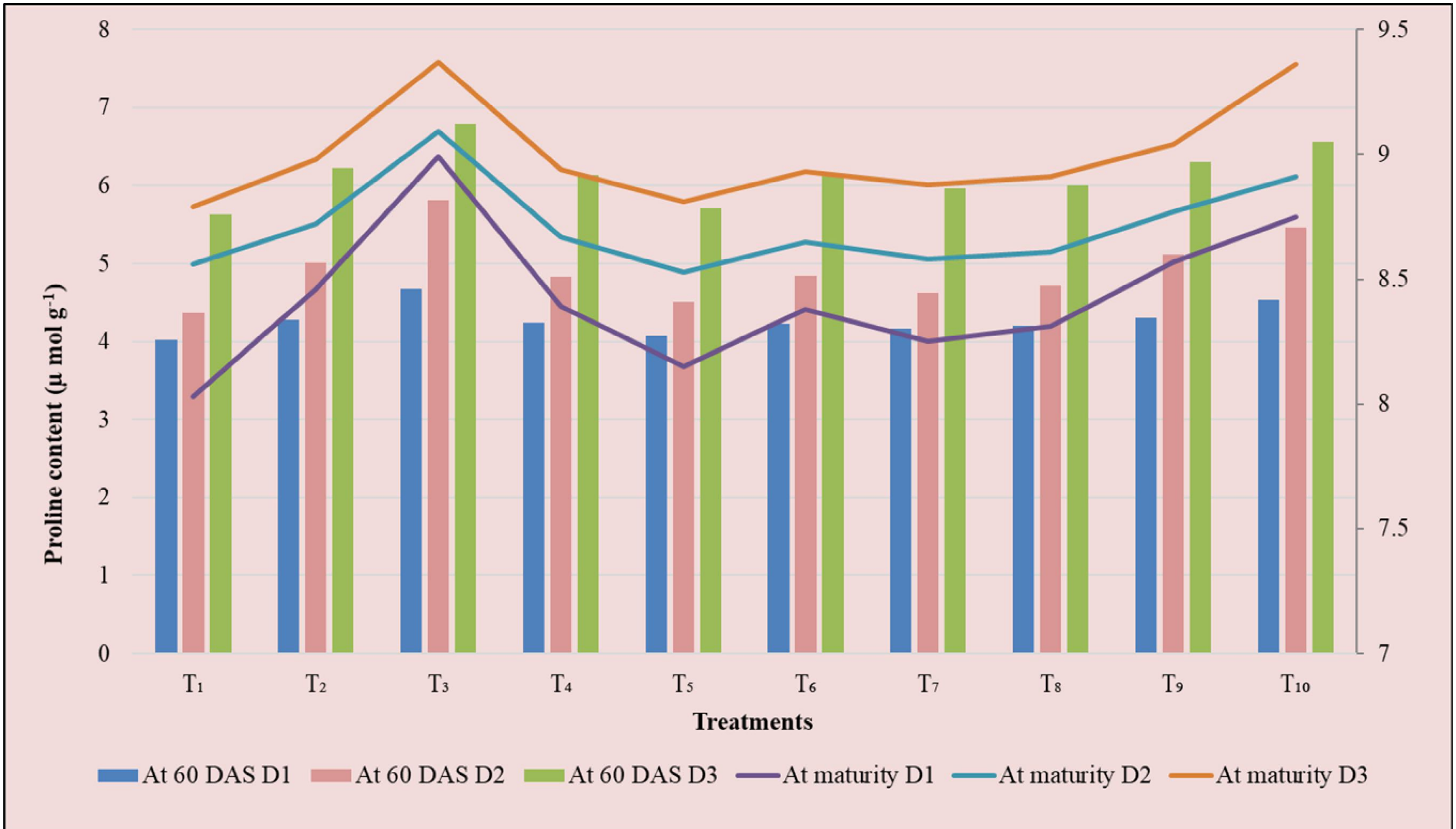

Fig. 3. Effect of dates of sowing and heat stress mitigating chemicals on proline content in chickpea

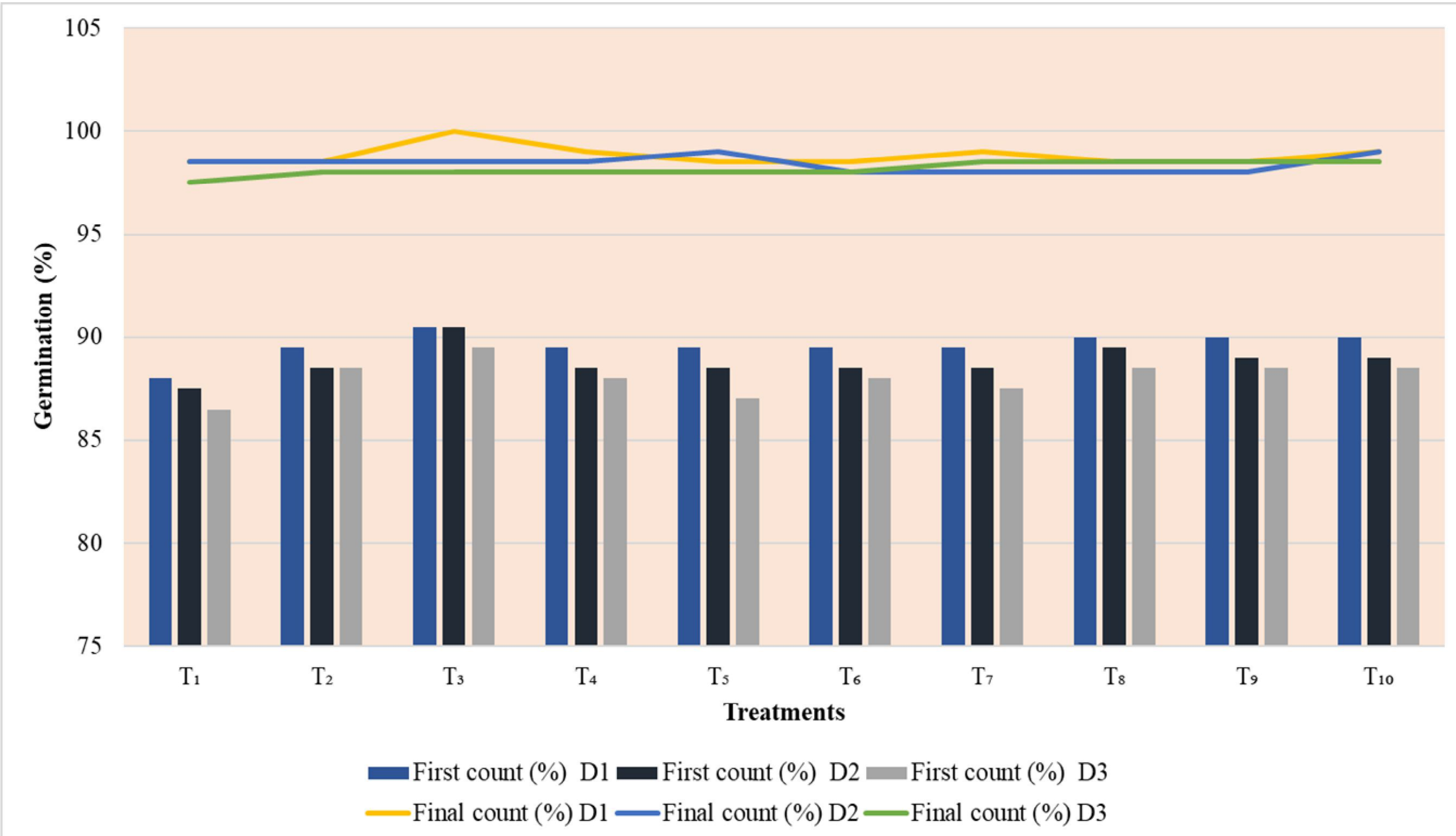

Fig. 4. Effect of dates of sowing and heat stress mitigating chemicalson germination (%) in chickpea

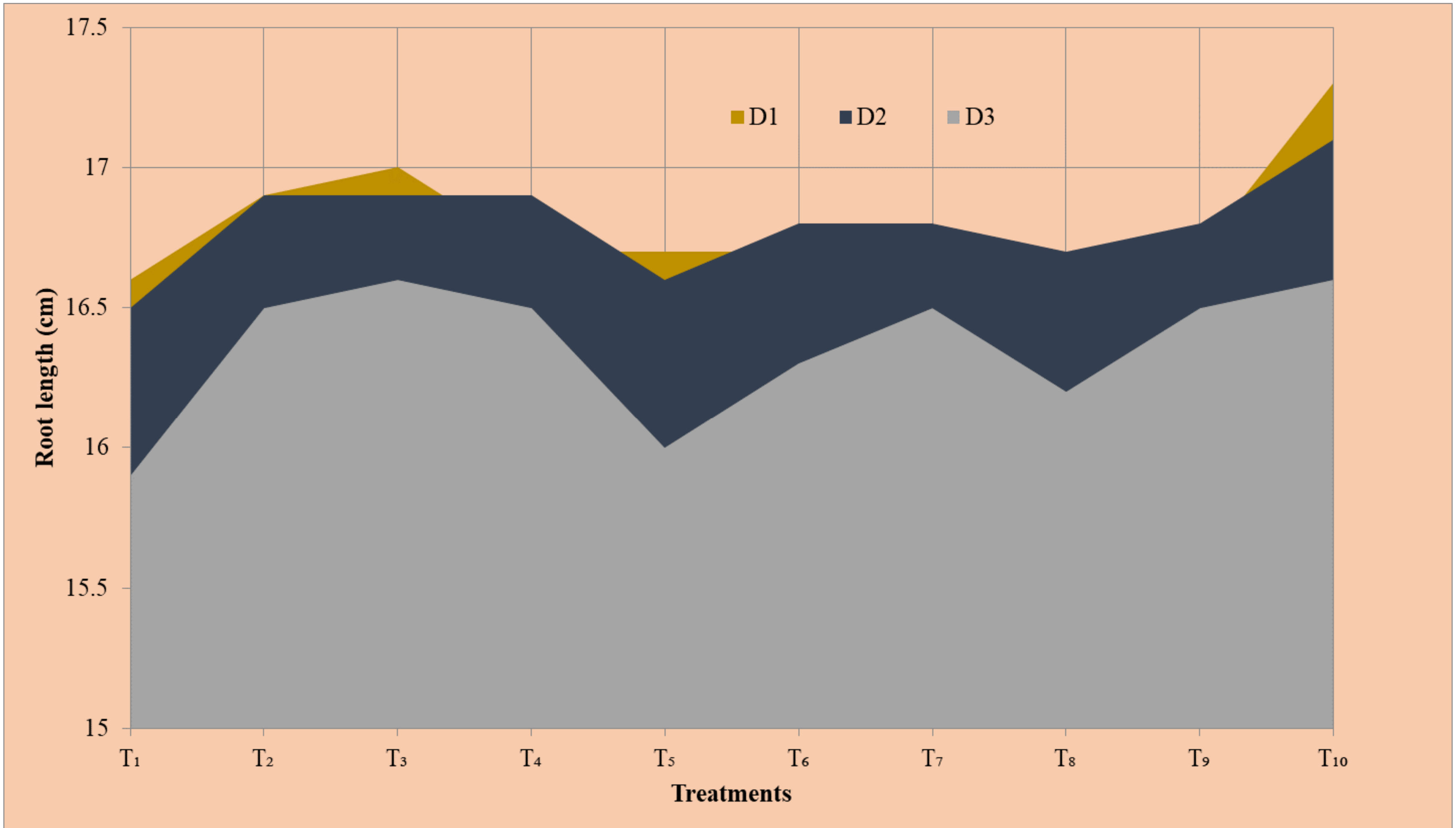

Fig. 5. Effect of dates of sowing and heat stress mitigating chemicals on root length in chickpea

Fig. 6. Effect of dates of sowing and heat stress mitigating chemicalson shoot length in chickpea

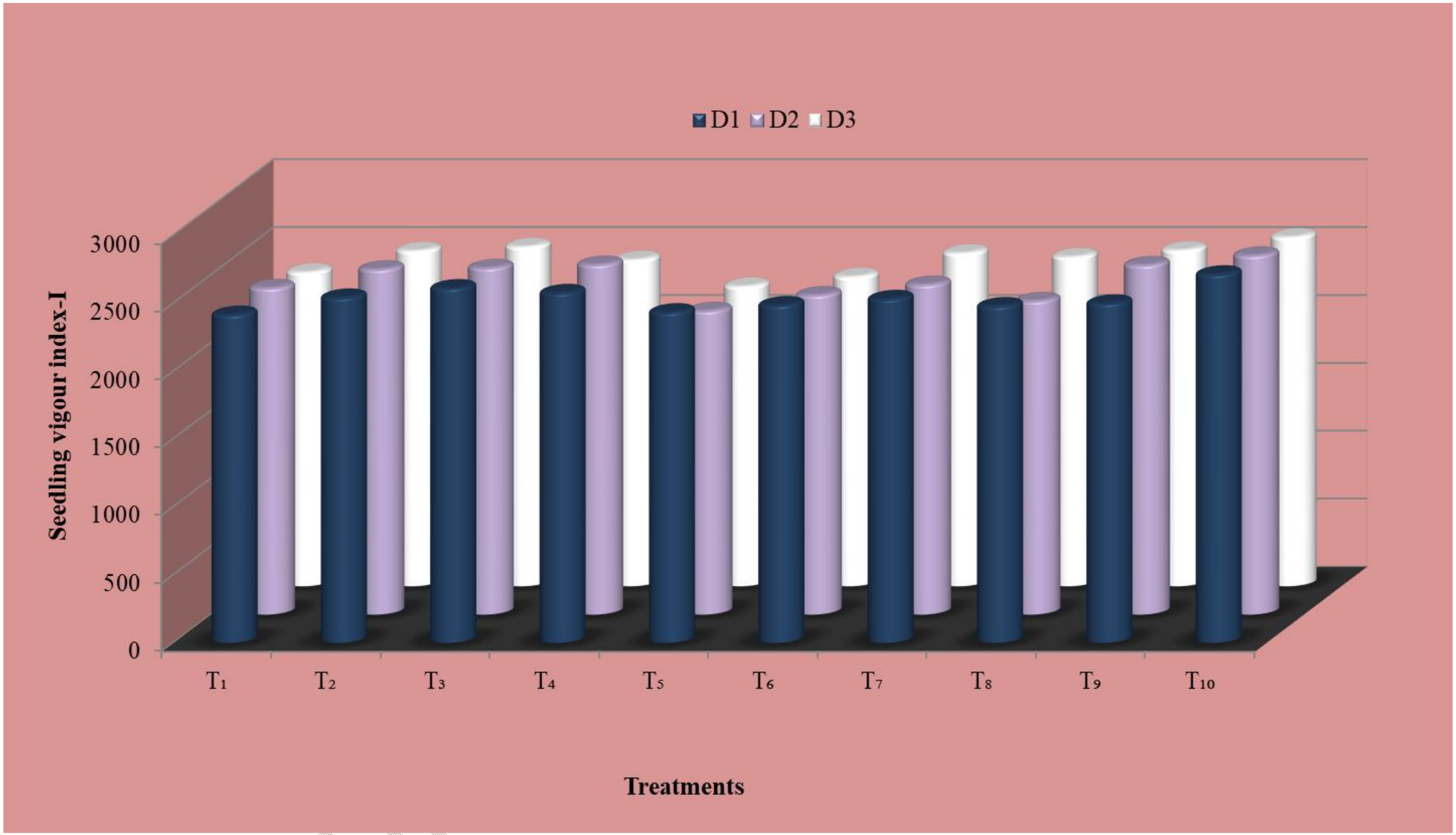

Fig. 7. Effect of dates of sowing and heat stress mitigating chemicals on seedling vigour index-I in chickpea

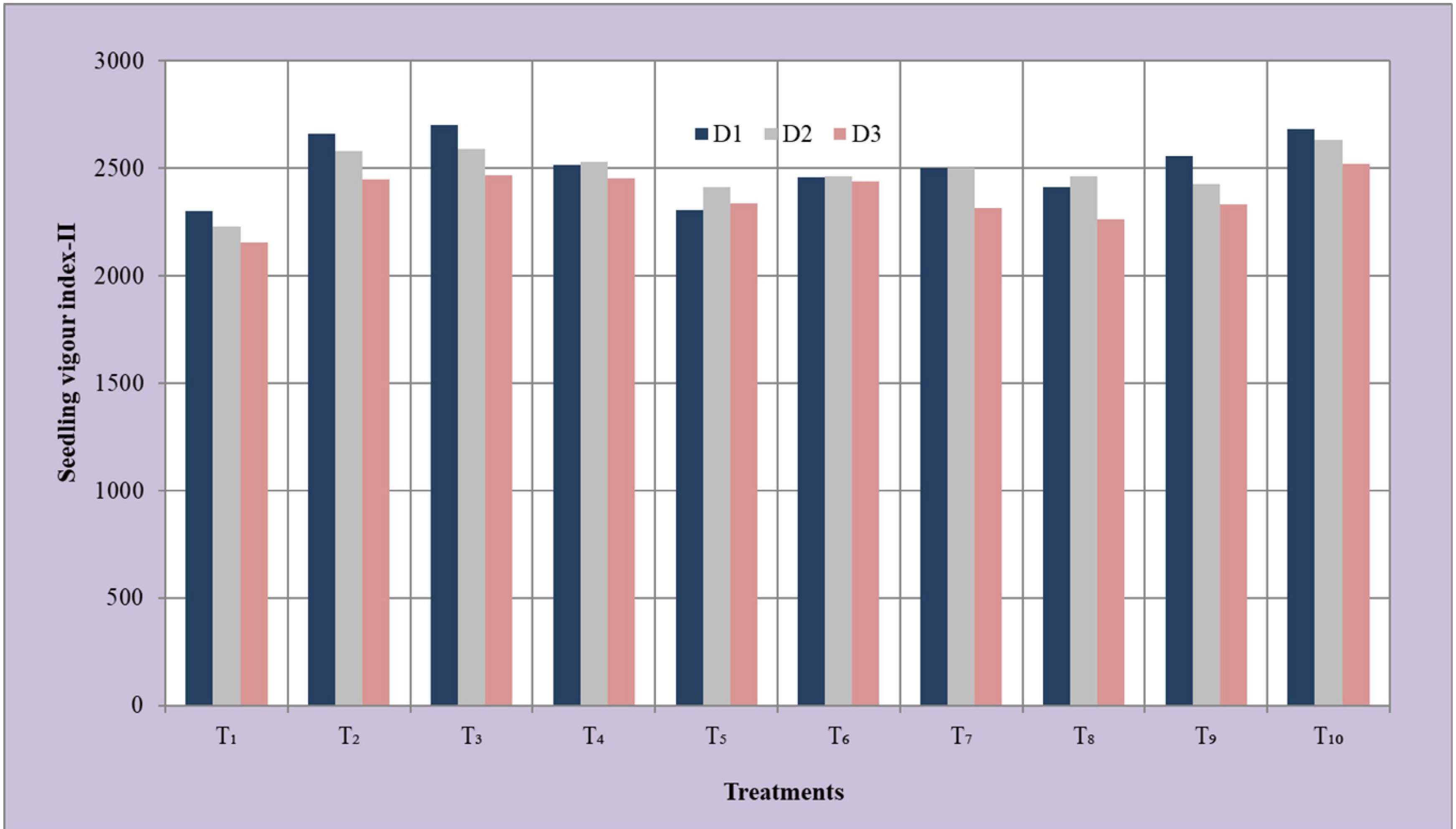

Fig. 8. Effect of dates of sowing and heat stress mitigating chemicalson seedling vigour index-II in chickpea

Normal sowing (D_1) Late sowing (D_2) Very late sowing (D_3)

Plate 1: General view of experimental plot

At 60 DAS

At maturity

Normal sowing

At 60 DAS

At maturity

Very late sowing

Plate 2: Effect of dates of sowing and heat stress mitigating chemicals on proline content

D_1T_3 : Normal sowing + Salicylic acid @ 400 ppm

D_1T_1 : Normal sowing + Control

D_3T_3 : Very late sowing + Salicylic acid @ 400 ppm

D_3T_1 : Very late sowing + Control

Plate 3: Effect of dates of sowing and heat stress mitigating chemicals on seed germination

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D_1T_{10} : Normal sowing + Gibberellic acid @ 100ppm

D_1T_1 : Normal sowing + Control

D_3T_3 : Very late sowing + Gibberellic acid @ 100ppm

D_3T_1 : Very late sowing + Control

Plate 4: Effect of dates of sowing and heat stress mitigating chemicals on seedling shoot and root length

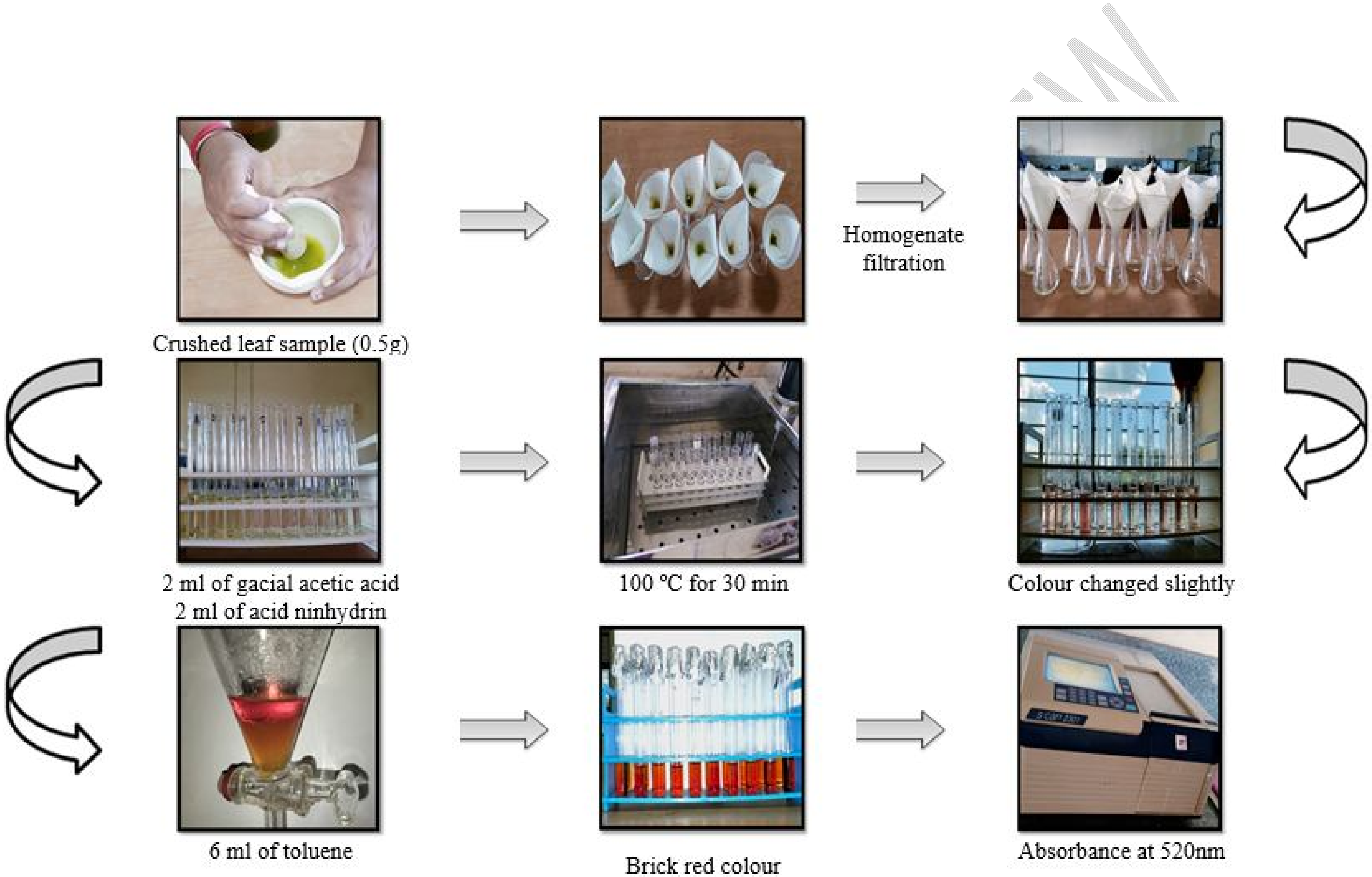
Crushed leaf sample (0.5g)
Homogenate
filtration
2 ml of gacial acetic acid
2 ml of acid ninhydrin
100 °C for 30 min
Colour changed slightly
6 ml of toluene
Brick red colour
Absorbance at 520nm

Plate 5. Procedure for estimation of proline content

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Plate 6. Procedure for estimation of relative water content

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