Journal of Advanced Medical and Dental Sciences Research

@Society of Scientific Research and Studies ***NLM ID:*** *101716117*

Journal home page: www.jamdsr.com doi: 10.21276/jamdsr Indian Citation Index (ICI) Index Copernicus value = 91.86

(e) ISSN Online: 2321-9599; (p) ISSN Print: 2348-6805

Original Research

Morpho-Physiological and Biochemical Characteristic in Seedlings of *Cicer Species* in response to Chilling Stress on

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ABSTRACT:
Low temperature is one of the abiotic stress that affects the survival, growth and reproduction of crop plants. The present investigation was carried out to study the influence of chilling stress of 0°C and 4°C for 24hr and 48hr on germination potential, radicle length, plumule length and moisture content of seedlings after different intervals of time in *cicer arietinum.* The level of various biochemical reserves of seedlings was also studied. All the above morphological, physiological and biochemical parameters were significantly influenced by the various chilling stress treatments as compared to control. The increase was due to cellular and metabolic changes that occurred during cold acclimation.
Key Words: Seed size, germination, chilling stress and seedling vigour.

Received: 23 April, 2022 Accepted: 28 May, 2022

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This article may be cited as: Sidhu HK. Morpho-Physiological and Biochemical Characteristic in Seedlings of Cicer Species in response to Chilling Stress on. J Adv Med Dent Scie Res 2022;10(6):101-105.

INTRODUCTION

Plants are exposed to considerable fluctuations in temperature during day-night cycle and at changes in weather conditions and seasons. Each plant species have evolved a degree of tolerance to temperature. Chilling temperature is injurious to plants of tropical and sub-tropical origin where they are exposed to low positive temperature. Chilling effect is exhibited by physiological agitation, a phenomenon known as chilling injury (Yan *et al.,* 2010). The genus ***cicer*** includes about 40 species. Many factors affect growth and development of plant. The planting value of seed is the ultimate objective of seed quality. Germination test is the most widely used test for seed viability. Seed vigour is the end result of various properties that determine potential for rapid, uniform emergence and development of healthy seedlings. Various external and internal factors affect the morphological, physiological, biochemical and anatomical parameters. Among these factors chilling stress have been shown to have profound effect on various morpho-physiological and biochemical parameters of seedlings.

MATERIALS AND METHODS

The seeds of ***Cicer Species***. were procured from the Department of Plant Breeding of Punjab Agriculture University, Ludhiana (Punjab) and the experiment was conducted in department of Botany, Desh Bhagat University, Mandi Gobindgarh. Seeds were hand separated and graded uniform seeds were surface sterilized with 0.1% mercuric chloride for one minute, followed by thorough washing with distilled water. The seeds were soaked in distilled water in beakers and kept at 0°C and 4°C for 24hrs and 48hrs respectively. The seeds were then sown in sterilized periplates in three replications. One set of petriplates containing seeds were kept as control which were not given any chilling stress. The petriplates were kept in seed germinator at 10% relative humidity. Data was recorded on various morphological parameteres like radicle length and plumule length of seedlings after different intervals of time i.e. 24hrs. to 192hrs. Physiological parameters include fresh weight, dry weight and moisture content of the seedlings. Biochemical reserves like total soluble sugars, starch (Dubois *et al.,* 1956) and total soluble proteins (Lowry *et al.,* 1951) were also estimated in seedlings.

MORPHOLOGICAL PARAMETERS

GERMINATION PERCENT

The number of seeds germinated after different intervals of time were counted in control as well as in chilling stress treated petriplates.

$$\text{Germination Percent} = \frac{\text{No.of seeds germinated}}{\text{Total No.of seeds sown}} \times 100$$

Radicle Length and Plumule Length (cm)
It was determined with the help of scale.

Physiological Parameters

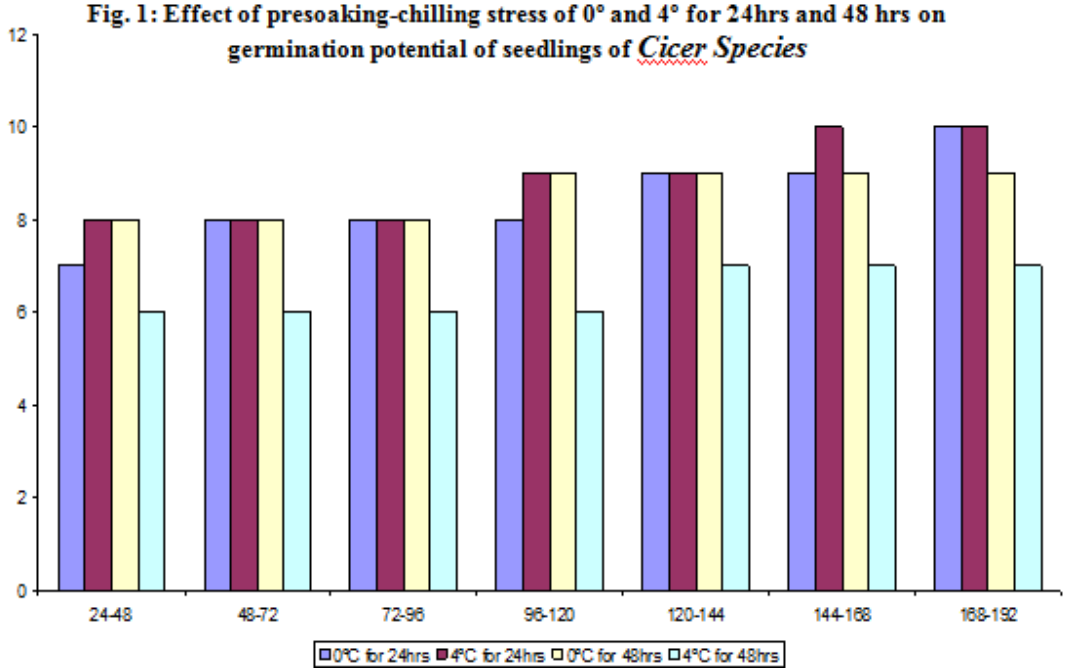
Fresh Weight, Dry Weight and Moisture Content of Seedlings
Fresh weight of the seedlings was taken after different intervals of time by weighing them on a balance and for dry weight estimation, the same seedlings were kept in oven at 100ºC.

$$\text{Percent Moisture Content} = \frac{\text{Fresh Weight - Dry Weight}}{\text{Dry Weight}} \times 100$$

RESULTS AND DISCUSSION

Fig. 1 shows that the germination potential was significantly effected by various presoaking chilling treatments. All the chilling treatments caused significant increase in the germination of the seeds. Maximum increase was with 4ºC for 48hrs. This data is similar to the findings reported in chickpea (Abha Chohan, 2011).

Fig. 1: Effect of presoaking-chilling stress of 0° and 4° for 24hrs and 48 hrs on germination potential of seedlings of *Cicer Species*



RADICLE LENGTH AND PLUMULE LENGTH (CM) OF THE SEEDLINGS

Table 1 and Table 2 show that effect of chilling stress on radicle length and plumule length of the seedlings.

Table 1: Effect of presoaking chilling stress of 0°C and 4°C for 24hr and 48hr on radicle length (cm) in seedlings of *Cicer Species*

Time interval (hrs.)	Control	0°C for 24 hr.	0°C for 48 hr.	4°C for 24hr.	4°C for 48hr.
24-48	-	1.8±0.5	2.0±0.7	17±0.7	1.6±0.9
48-72	1.8±0.5	2.9±1.3	3.8±0.8	2.7±0.3	2.0±0.3
72-96	2.0±0.8	3.5±1.8	4.9±0.8	3.0±0.7	2.9±0.9
96-120	2.9±1.3	4.9±0.9	6.3±0.7	4.4±0.9	4.2±0.7
120-144	3.1±1.8	6.4±1.9	8.7±0.8	6.3±0.7	6.9±0.9
144-168	4.3±0.9	7.8±1.9	13.2±1.9	7.0±1.0	7.4±1.2
168-192	5.0±1.8	9.4±0.9	18.5±1.8	90. ±0.9	9.2±0.9

Significant at 5% level; Values represent mean ± S.E.

There was marked enhancement in the radicle length of seedlings given chilling stress at different intervals of time as compared to control. The seedlings which were subjected to stress of 0°C for 24hrs. had significant enhancement in length of radicle as compared to seedlings subjected to stress of 0°C for 48hours. Likewise chilling stress of 4°C for 24hrs also caused significant enhancement in radicle length of the seedlings but % age increase was less than that of 0°C treatment (Table 1).

Table 2: Effect of presoaking chilling stress of 0°C and 4°C for 24hr and 48hr on plumule length (cm) in seedlings of *Cicer Species*

Time interval (hrs.)	Control	0°C for 24 hr	0°C for 48 hr	4°C for 24hr	4°C for 48hr
24-48	-	0.95±0.03	0.68±0.01	0.52±0.08	0.40±0.09
48-72	1.62±0.01	2.06±1.30	1.23±0.01	1.02±0.02	0.09±0.01

72-96	1.85±0.18	4.16±0.20	4.06±0.10	2.36±0.08	1.36±0.03
96-120	2.93±0.08	5.32±1.20	5.02±0.18	3.90±0.17	2.86±0.08
120-144	5.02±1.80	6.35±0.08	6.20±0.18	5.03±0.08	4.96±0.18
144-168	6.06±0.08	7.20±0.09	6.90±1.8	5.65±0.08	5.36±0.08
168-192	6.95±0.09	81±0.08	7.13±0.09	6.01±0.08	5.93±0.07

Significant at 5% level; Values represent mean ± S.E.

Similar trend was observed in plumule length of seedlings (Table 2). There was marked reduction in radicle length and plumule length of seedlings subjected to chilling stress of 4°C for 48hrs. This reduction was due to stress caused marked changes in the morpho-physiological characteristics of seedlings (Singh, K.B. 1990).

PHYSIOLOGICAL PARAMETERS

Fresh Weight, Dry Weight and Moisture Content also showed marked variation with various stress treatments. Table 3 shows that there was significant increase in the fresh weight of seedlings at 0ºC for 24, 48hrs and 4ºC for 24hrs, but there was reduction in the fresh weight following stress treatment at 4ºC for 48hrs. The dry weight was also affected in the similar trend. Moisture content was significantly higher in chilling stress treated seedlings as compared to control. Fresh weight (FW) is related to tissue volume, decrease in fresh weight represents increase in the leaf thickness. Decrease in dry weight indicate transient dry matter accumulation and for increased cell wall thickness (LU, Z. and Neumann, P.M. 1999).

Table 3: Effect of pre soaking chilling stress of 0ºC and 4ºC for 24hrs and 48hrs on fresh weight (g), dry weight (g) and moisture content (%) in seedlings of *Cicer arietinum*

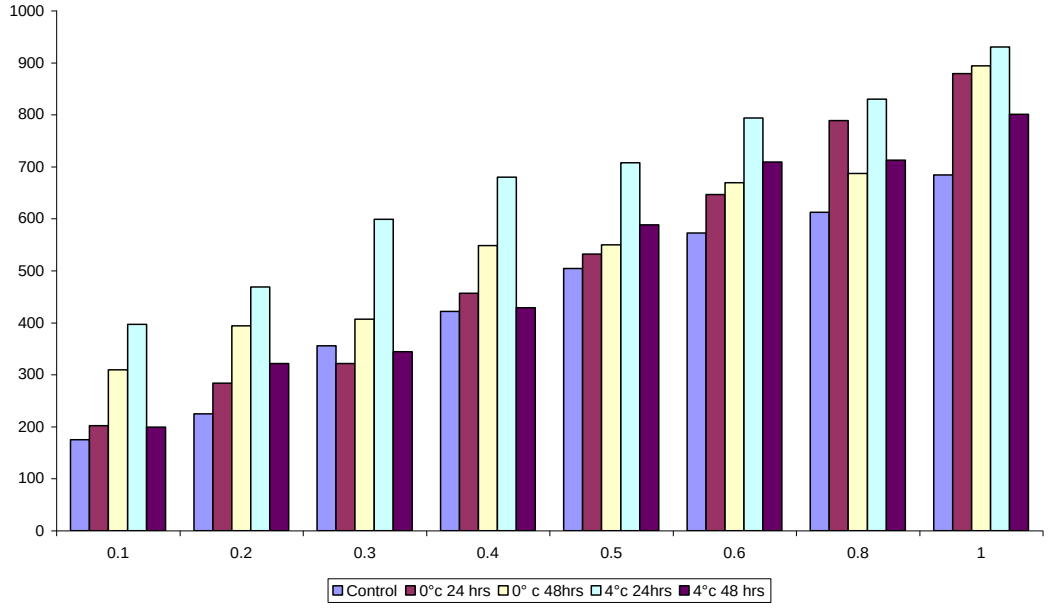
Time Internal	0°c for 24hrs			0°c for 28hrs			0°c for 24hrs			0°c for 28hrs		
	Fresh weight	Dry weight	Moisture content	Fresh weight	Dry weight	Moisture content	Fresh weight	Dry weight	Moisture content	Fresh weight	Dry weight	Moisture content
24-48	0.379 ±0.01	0.035 ±0.02	982.8	0.29± 0.04	0.010 ±0.02	2800	0.477 ±0.02	0.053 ±0.03	800	0.46± 0.01	0.019 ±0.03	2321.1
48-72	0.400 ±0.03	0.040 ±0.04	900	0.23± 0.02	0.012 ±0.01	1816.6	0.281 ±0.04	0.032 ±0.02	778.1	0.38± 0.04	0.014 ±0.02	2614.3
72-96	0.047 ±0.02	0.040 ±0.03	917.5	0.34± 0.01	0.023 ±0.03	13.8	0.312 ±0.01	0.034 ±0.04	817.6	0.45± 0.01	0.021 ±0.01	2042.8
96-120	0.449 ±0.01	0.036 ±0.01	1147.2	0.21± 0.03	0.011 ±0.04	1809.0	0.320 ±0.03	0.035 ±0.02	814.3	0.63± 0.04	0.024 ±0.04	2525
120-144	0.423 ±0.03	0.043 ±0.04	883.7	0.20± 0.02	0.014 ±0.02	1328.5	0.288 ±0.04	0.015 ±0.03	1820	0.41± 0.03	0.019 ±0.01	2057.9
144-168	0.470 ±0.04	0.041 ±0.02	1046.3	0.29± 0.04	0.020 ±0.04	1350	0.259 ±0.02	0.013 ±0.01	1892.3	0.63± 0.02	0.023 ±0.03	263.9.1
168-192	0.474 ±0.02	0.336 ±0.01	1216.6	0.36± 0.02	0.017 ±0.03	2017.6	0.394 ±0.03	0.044 ±0.04	795.4	0.54± 0.01	0.026 ±0.04	1976.9
192-216	0.441 ±0.01	0.046 ±0.03	858.7	0.35± 0.01	0.026 ±0.01	1246.2	0.474 ±0.01	0.048 ±0.03	887.5	0.48± 0.03	0.021 ±0.02	2185.7
216-240	0.451 ±0.01	0.035 ±0.01	1188.6	0.41± 0.04	0.011 ±0.02	3627.3	0.437 ±0.02	0.032 ±0.01	1265.6	0.44± 0.04	0.014 ±0.03	3042.8
240-264	0.578 ±0.04	0.037 ±0.02	1462.2	0.43± 0.02	0.031 ±0.04	1287.1	0.569 ±0.04	0.041 ±0.02	1287.8	0.43± 0.01	0.023 ±0.02	1769.6

Significant at 5% level, values represent mean ± S.E.

BIOCHEMICAL RESERVES

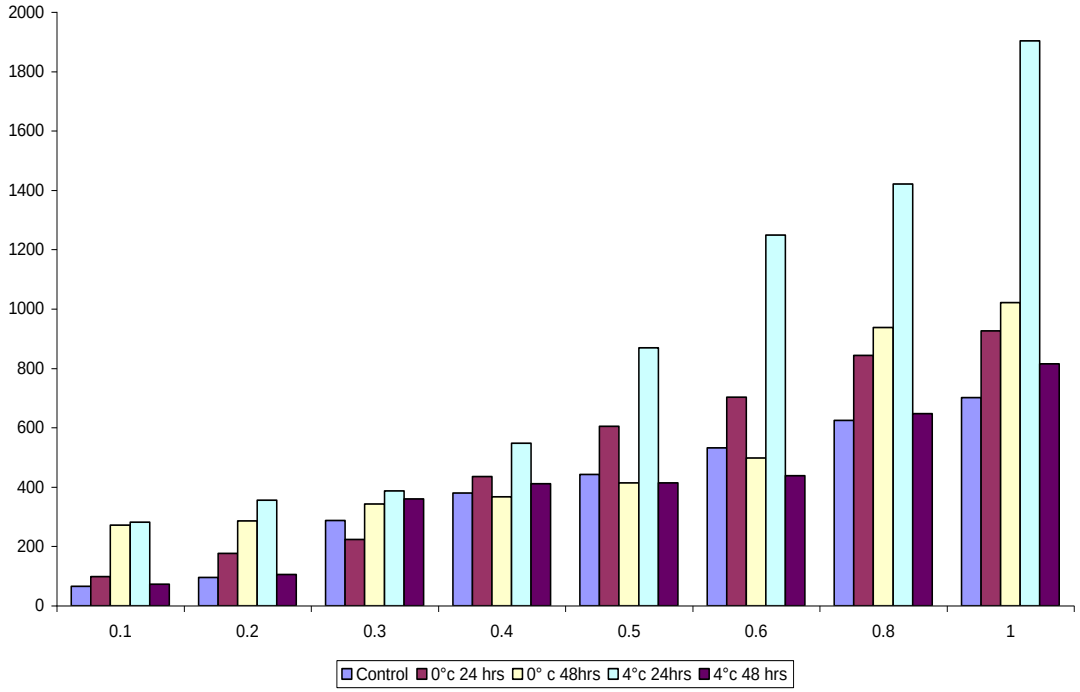
Chilling stress also resulted in marked variation in level of various biochemical reserves of seedlings like total soluble sugars, starch and total soluble proteins. Fig. 2, 3 & 4 showed that as compared to control, the level of all the reserves were significantly enhanced by chilling stress of 0°C for 24hrs. and 48hrs. and 4°C for 24hrs. But the stress of 4°C for 48hrs. caused a marked reduction in level of various reserves. The percentage increase in the level of total soluble sugars in case of seedlings given presoaking chilling stress of 0°C for 24hrs. 0°C for 48hr. and 4°C for 24hrs. was 4.51%, 2.78% and 0.95% respectively as compared to the control but the level showed a marked reduction in seedlings given stress treatments of 4°C for 48hrs (Fig. 2).

Fig. 2: Effect of presoaking-chilling stress of 0º and 4º for 24hrs and 48 hrs on total soluble sugars (□g seed-1) in seedlings of Cicer Species



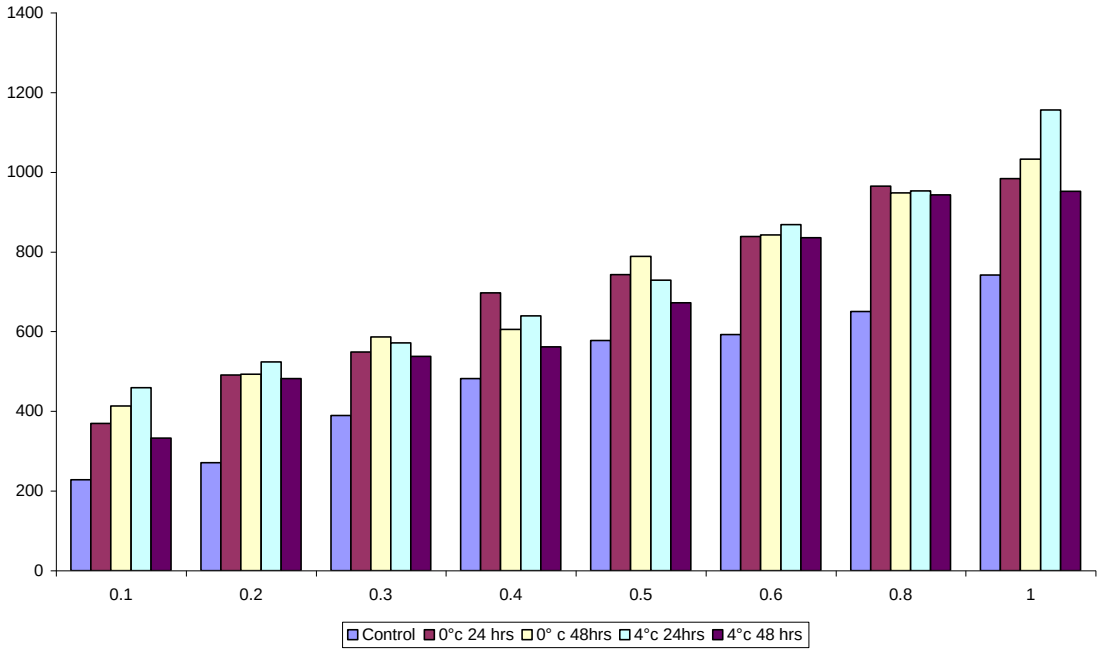
Similar trend was observed in the level of starch content (Fig. 3).

Fig. 3: Effect of presoaking-chilling stress of 0º and 4º for 24hrs and 48 hrs on total starch content (□g seed-1) in seedlings of Cicer Species



The protein content was significantly enhanced as compared to the level of total soluble sugars and starch, giving a clear indication that gram seeds are rich source of protein. The percentage increase in seedlings subjected to stress of 0°C for 24hrs, 0°C for 48hr and 4°C for 24hrs. was 30.5%, 25.41% and 15.77% respectively as compared to control but the seedlings given 4°C for 48hrs stress treatment showed a significant reduction in level of protein content as compared to control. (Fig. 4) Low temperature caused marked effects on the growth and total protein amount of chick pea (Munzuroglu O., et al., 2005). Similar effect of chilling stress on soluble proteins, sugars and proline accumulation was reported in cotton genotypes (Azymi S. *et al.*, 2012).

Fig. 4: Effect of presoaking-chilling stress of 0º and 4º for 24hrs and 48 hrs on total soluble proteins (□g seed-1) seedlings of Cicer Species



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