Effects of chilling on seed germination and early seedling growth of *Vigna radiata* (L.) Wilczek

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Abstract

The present investigation was carried out to find out the effect of chilling stress on the seed germination and seedling growth of *Vigna radiata* (L.) Wilczek. The experiments were conducted in Petri plates by using absorbent cotton. The seeds of *V. radiata* were kept for different time intervals (6h, 12h, 18h and 24h) in the freezer of a refrigerator at 4^0C and then the seeds were allowed to germinate in Petri plates lined with absorbent cotton. Control was also prepared by using non-stressed seeds. The different parameters like germination percentage and the seedling growth parameters like shoot length, fresh weight and dry weight of the seedlings were determined. From the present research work, it was found that the chilling treatments for 6h, 12h, 18h and 24h had no significant effects on the seed germination and early seedling growth of green gram. Even though the seedlings raised from 6h treated seeds showed some enhancement in the shoot length and fresh weight of seedlings it was not carried into the dry weight and dry weight percentage of seedlings.

Key words: Chilling stress, dry weight, fresh weight, germination, seedling

Introduction

Environmental stresses interfere with every aspect of plant growth and metabolism. The responses of plants towards these stresses depend upon various factors such as duration and degree of stress, growth stage and time of stress exposure etc. Due to their sedentary mode of life, plants resort to many adaptive strategies in response to different stresses such as high salt, dehydration, low temperature and heat, which ultimately affect the plant growth and productivity. Against these stresses, plants adapt themselves by various mechanisms like change in morphological and developmental pattern as well as physiological and biochemical responses.

Temperature is one of the major environmental factors affecting germination (Nykiforuk and Johnson-Flanagan 1994). Because temperature is one of the most effective factors which control seed germination, therefore, any unusual temperature changes will interrupt the normal series of seed germination and result in dropping the ability of seeds germination. Chilling is an important environmental stress that causes economic damage on crops and limiting the distribution of many crop species. In majority of plants, chilling injury occurs at a temperature range of 0-10^{o}c and it normally leads to damage in all stages of growth and development in the plant life cycle. Thus chilling stress surely interferes with the normal seed germination process of plants.

Seeds are the primary and essential starting point for a wide range of agronomic important plants. Because of the interest and demand to improve crops, there is considerable

interest in increasing their germination ability. However, the increase in germination percentage seems to be greatly dependent on genotype, environment, cultivated treatments and their interactions (Kurt 2010). Temperature is one of the most critical factors affecting the germination of seeds. Low temperature induces dormancy in some cases, but in some species they are stimulatory especially within the range -1^0c to 15^0c. Improper freezing can kill some seeds but other seeds may not get harmed. In fact, many wild flower, tree and shrub seeds actually require a cold period or stratification, before they will germinate. Chilling stress induces the leakage of intracellular electrolytes from tissues as a result of the loss of cytoplasmic membrane integrity (Borowski *et al*. 1997; Borowski and Blamowski 2009).

In the present research work the effects of chilling stress on the seed germination and early seedling growth of *Vigna radiata* (L.) Wilczek was investigated by analyzing the germination percentage and the various growth parameters of the seedlings.

Materials and methods

Plant material

The research was carried out with the seeds of *Vigna radiata* (L.) Wilczek were procured from stores at Kodungallur.

Methods

Chilling treatment

Healthy and plumby seeds of *V. radiata* were washed with 0.1% mercuric chloride, detergent solution and distilled water to remove any dirt present on the seed surface. The washed seeds were surface dried by using filter paper. These seeds were put in separate paper bags each bags containing 50 seeds. All the paper bags were placed in the freezer of a refrigerator and maintained the temperature 4^0C inside the freezer. Theses seeds were exposed for different time periods (6h, 12h, 18h and 24h) and after each time period, the seeds were taken out from the freezer. Then the seeds were used for germination studies.

Incubation of seeds in the Petri dishes

The treated seeds (10 numbers) along with untreated (control) seeds were allowed to germinate in Petri dishes containing absorbent cotton soaked with distilled water. All the Petri dishes were kept in the culture racks under controlled light and temperatures. Seeds were allowed to germinate and examined regularly for the data collection.

Determination of Germination percentage

Percentage of germination was calculated by using the formula,

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

Determination of shoot length, fresh weight, dry weight, dry weight percentage and moisture content percentage.

Shoot length of seedlings were measured by using scale. Measurements were taken in seedlings which were nine days old. The seedlings were weighed using electronic balance. For fresh weight and dry weight measurements, the seedlings were blotted and wrapped separately in pre-weighed labelled aluminium foils. Fresh weight of the samples was determined by weighing them immediately after wrapping. For dry weight measurements the samples were kept in a hot air oven at 100^0 C for one hour followed by at 60^0C for overnight. After 48 h, the samples were transferred to a desiccator, allowed to cool and then weighed. The samples were reweighed as described above at regular intervals (24h), until the weights became constant. The dry weight percentage was calculated by using the following formula:

$$\text{Dry weight percentage} = \frac{\text{Dry weight}}{\text{Fresh weight}} \times 100$$

Moisture content percentage was calculated by using the following formula

$$\text{Moisture content percentage} = \frac{\text{Fresh weight - Dry weight}}{\text{Fresh weight}} \times 100$$

Statistical analysis

The results were analysed by using Microsoft excel. Standard deviation and standard error were determined for each category of data.

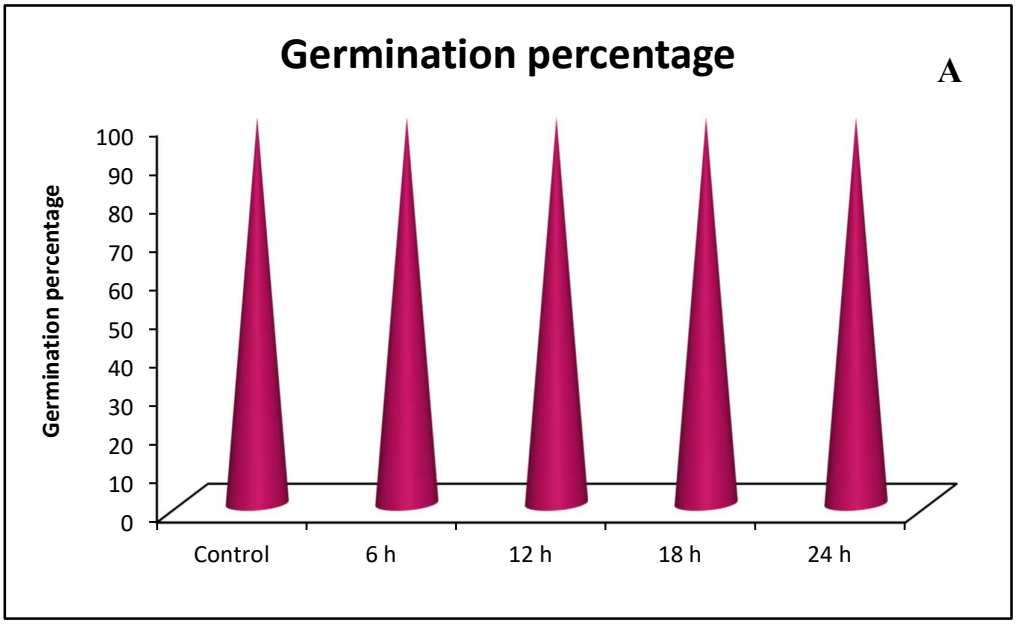
Results

Germination percentage

As far as the germination percentage of seeds were concerned, we got 100% seed germination in control and in all the other treatments (6h, 12h, 18h and 24h). The germination of seeds was completed in the fourth day itself in all the Petri plates (Fig. 1A).

Shoot length, fresh weight, dry weight, dry weight percentage and moisture content percentage

Among the different treatments, shoot length, fresh weight and moisture content percentage were found to be maximum in the seedlings which were raised from the 6h treated seeds in 4^0C. The maximum recorded shoot length of seedlings was 18.5cm and fresh weight was 0.332g in the seedlings raised from 6h treated seeds. While the minimum shoot length recorded was 14cm in the seedlings which were raised from 18h treated seeds and the minimum fresh weight recorded was 0.115g in the seedlings raised from control seeds. But the dry weight (0.042g) and dry weight percentage (36.5%) were found to be maximum in the control seedlings. Moisture content percentage was found to be more in treatments when compared to control seedlings (Fig. 1B, 2A, 2B, 3A, 3B).



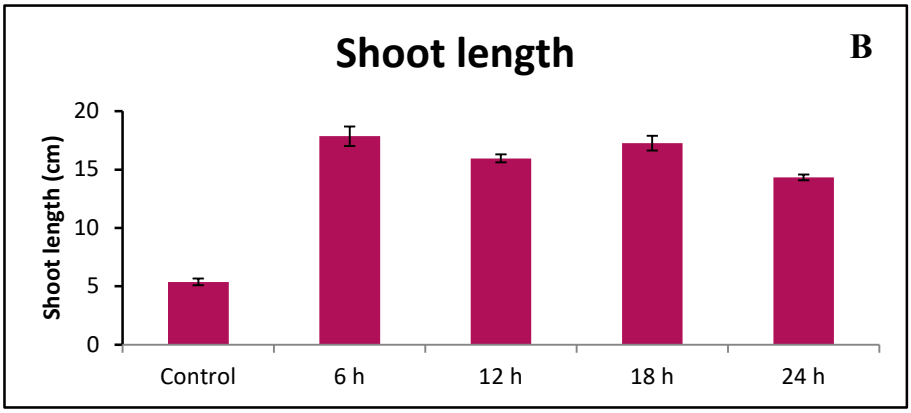


Fig.1: Germination percentage (**A**) and Shoot length (**B**) of *Vigna radiata* L. Wilczek seedlings raised from control and chilled seeds (6h, 12h, 18h, 24h). The vertical bars represent SE of the mean value of recordings from three independent experiments each with a minimum of three replicates.

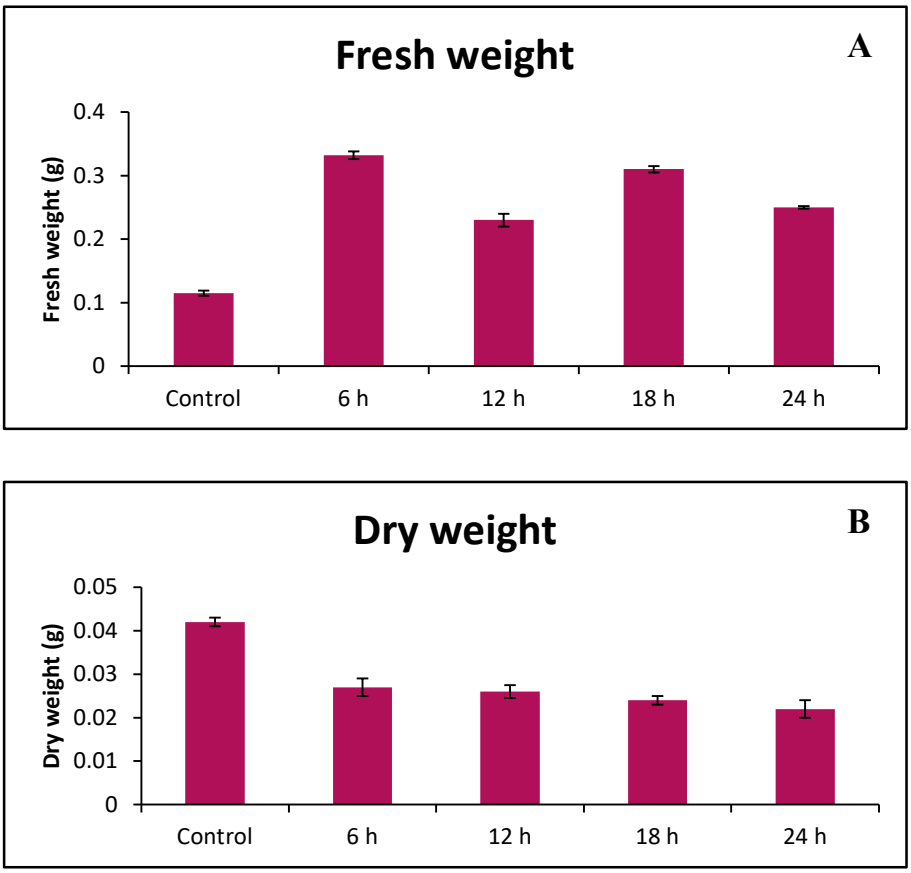


Fig.2: Fresh weight (**A**) and Dry weight (**B**) of *Vigna radiata* L. Wilczek seedlings raised from control and chilled seeds (6h, 12h, 18h, 24h). The vertical bars represent SE of the mean value of recordings from three independent experiments each with a minimum of three replicates.

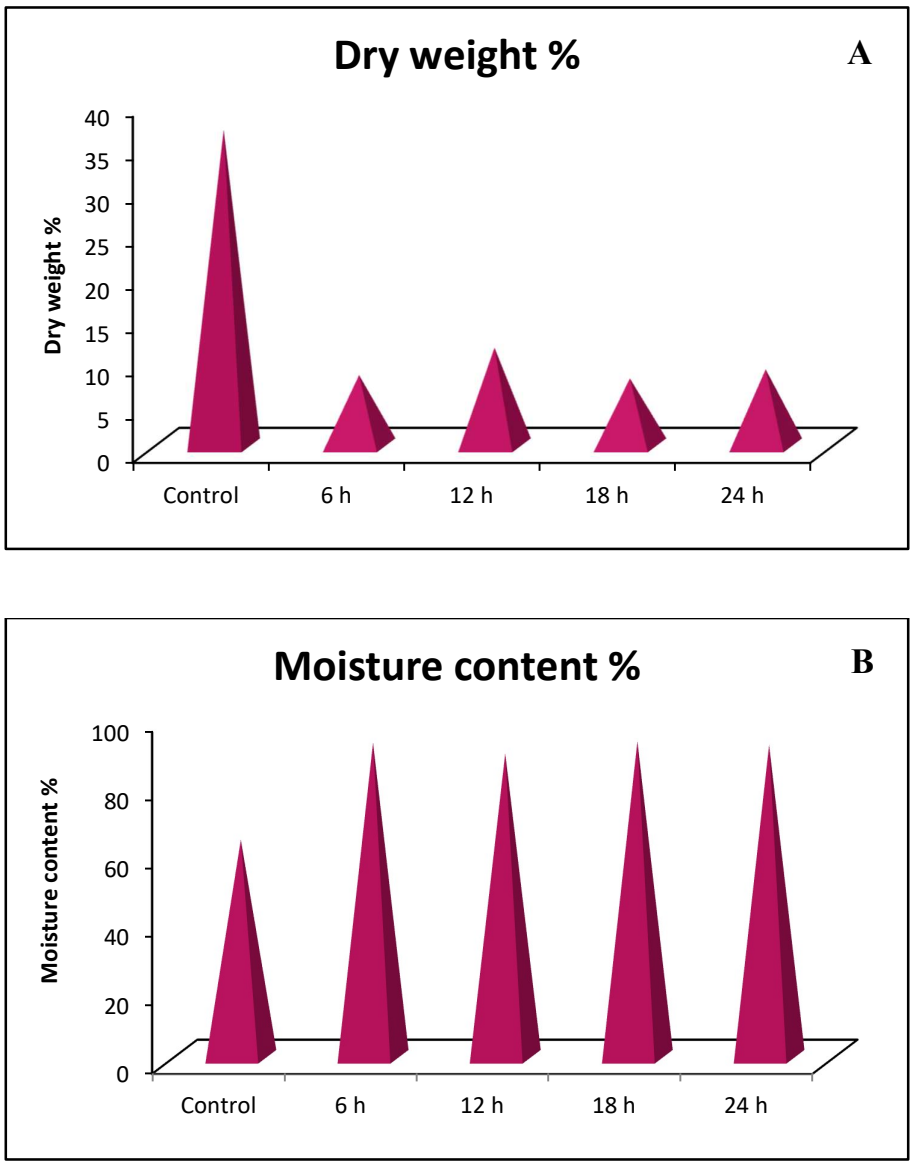


Fig.3: Dry weight percentage (**A**) and Moisture content percentage (**B**) of *Vigna radiata* L. Wilczek seedlings raised from control and chilled seeds (6h, 12h, 18h, 24h). The vertical bars represent SE of the mean value of recordings from three independent experiments each with a minimum of three replicates.

Discussion

During the past few decades much research had been carried out in the field of various environmental stress and their affects on plants. Among these stresses, chilling stress is an important factor which causes many deleterious effects on plant growth and development. Interestingly chilling stress also poses some positive impacts on plants which include increased seed germination. The present research was carried out to know whether low temperature like 4^0C had any positive or negative impacts on the seed germination and early seeding growth of *V. radiata*. The results of the study showed that in

the case of green gram seeds the 4^0C had no significant role in seed germination under the different time intervals. This may be due to the decreased time period that we had provided or may be due to the less impact of chilling stress. There were many studies regarding the chilling stress and its effects on germination in many crops like *Gossypium* (Anjum and Khatoon 2003), *Sinapis arvensis* L. (Paolini *et al*. 2001), canola (Nykiforuk and Johnson-Flanagan 1994) etc. According to Kurt (2010) in flax, the seed germination was enhanced by chilling treatments and it was also proved that the chilling period more effected than chilling temperature on germination of cultivar of flax.

There were seldom reports on the effects of chilling on the early seedling growth of plants. Most of the studies were focussed on the seed germination aspects only and the studies showed the positive effects of chilling treatments on seed germination. According to Baskin *et al*. (2001), chilling treatments break the dormancy of viable seeds and enhance germination in many species. In the present study, the seedling growth was also found to be varied under different treatments. As far as the shoot length and fresh weight of seedlings were concerned, it was found to be higher in the seedlings which were raised from treated seeds when compared to control seedlings. This indicates that even though the chilling stress did not cause any enhancement in seed germination, it slightly increased the initial seedling growth. But as far as the dry weight and dry weight percentage was concerned, it was found to be higher in control plants and it implies that the enhanced fresh weight is due to the increased water content in the seedlings and is evident from the moisture content percentage data of seedlings.

Summary and conclusion

The present investigation was carried out to find out the effect of freezing on the seed germination and seedling growth of *Vigna radiata* (L.) Wilczek. The seeds were subjected to 4^0C for different time intervals and the germination percentage and seedling growth parameters were analysed. From the present research work, it was found that the chilling treatments for 6h, 12h, 18h and 24h had no significant effects on the seed germination and early seedling growth of green gram. Even though the seedlings raised from 6h treated seeds showed some enhancement in the shoot length and fresh weight it was not much evident and was not observed in the dry weight and dry weight percentage parameters. Thus it was clearly demonstrated that chilling stress of 4^0C for 6h-24h had no significant effects on seed germination and early seedling growth of *Vigna radiata* (L.) Wilczek.

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