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A Novel Method of Seed Germination and Growth of Three Staple Crop Plants: Effect of Low Temperature and Cyanobacterial Culture Addition

Maya Khushwaha and Meenakshi Banerjee*,

Laboratory of Algal Biotechnology, Department of Bioscience, Barkatullah University, Bhopal, 462026, (M.P), India
*Department of BioSciences, Rice University, Houston, TX. USA
Correspondence: E-mail: minakshi12@aol.com

Abstract
The present study is an attempt to understand the effect of low temperature and presoaking with cyanobacterial extract on the seed germination of three important crop plants. Exposure of the seeds to low temperature treatment of 10 ° C for 5 days under laboratory conditions before potting the plants greatly stimulated the growth rates measured in terms of parameters like shoot and root length, number of leaves, Chlorophyll a and b content of leaves. These values` were greater than plants which were exposed to 25 ° C before potting. Addition of cyanobacterial culture to germinating seeds instead of water further enhanced the germination rate obtained with low temperature and this stimulatory effect was maintained in the potted plants. These results point to stratification/vernalization being involved in germination process. Stimulation of the germination with addition of cyanobacterial cultures emphasizes their hormonal role, as these organisms are known to liberate bioactive compounds into their growth medium which are very similar to plant growth hormones. Pretreatment with low temperature and cyanobacterial addition may be key to shortening the life cycle of these important crops and prove to be a very efficient way of managing crops to produce better and faster and therefore of great agricultural importance.

Key words: Vernalization, Cyanobacteria, Germination.

Introduction

Agriculture in India has a long history dating back to ten thousand years. High yields from crops and good quality products can be achieved only when the seeds are selected and sown wisely. Also soil plays a vital role in the efficiency of crop production and fertility is a big factor to be considered. For this it is necessary to test soil to find out what needs to be added to optimize them. Seeds are a vital component of the world's diet (Bewley, 1997). Seed germination depends on both internal physiological and metabolic conditions and external environmental conditions (Rangaswamy 1996).The external environment mainly the soil can be manipulated in several ways to make it a more productive for growth of plants. Among the most promising ways of doing this is the addition of nitrogenous and phosphatic fertilizers but it has its own disadvantages because they are environmentally damaging. Also the widespread application of single element fertilizers (especially N in Asian countries) in the cultivation of major crops has led to accelerated exhaustion of other major and minor nutrients leading to nutrient imbalances and poor soil fertility. On the other hand, cyanobacteria which are a group of prokaryotic photosynthetic and nitrogen fixing microorganisms are the most promising alternate biofertilizers being considered in replacement of the chemical ones worldwide today. De (1939) attributed the natural fertility of flooded rice field soil and its maintenance to the process of biological nitrogen fixation by cyanobacteria. This was the first report which recognized the agronomic potential of cyanobacteria in India. In addition to providing nitrogen, cyanobacteria also have phosphatase enzymes which break up the organic phosphorus in the soils to available inorganic forms. Thus they are considered as the most potent duel biofertilizers for crops today providing both nitrogen and phosphorus.

Cyanobacteria also possess a tremendous potential for producing a wide range of secondary metabolites and is a rich sources of biologically active constituent molecules such as auxins and Gibberellins (Fish & Codd 1994, Schlegel *et al*. 1999 Zaccaro *et.al.,* 2006). Production of secondary metabolites are linked to bio control of bacterial and fungal diseases as well as improving soil structure and porosity through secretion of polysaccharides aiding in soil aggregation are the most important functions of these microorganisms (Karthikeyan *et al*. 2007; Sergeeva *et al*. 2002; Banerjee and Sarkar 2008). As yet few studies have been conducted on the substitution of chemical fertilizers by

cyanobacterial biofertilizers. Gupta & Shukla (1967) studied the algal influence on growth, yield and protein content of rice plants and showed that pre-soaking rice seeds with BGA cultures or extracts enhances germination, promotes the growth of roots and shoots, and increases the weight and protein content of the grain. Svircev *et al*. (1997) Banerjee and Modi (2010), Banerjee *et al* (2010) also reported that plant growth was enhanced in the presence of cyanobacterial extracts and, even without organic N fertilizer application (Banerjee 1991; Banerjee and Kumar,1992; Banerjee 2003; Banerjee *et al* 2007**;** Banerjee 2007; Banerjee and Sarkar 2008).

Vernalization, is the artificial exposure of plants (or seeds) to low temperatures in order to stimulate flowering or to enhance seed production. By satisfying the cold requirement of many temperate-zone plants, flowering can be induced to occur earlier than normal or in warm climates lacking the requisite seasonal chilling. In the history of agriculture farmers observed a traditional distinction between "winter cereals," whose seeds require chilling, and "spring cereals," whose seeds can be sown in spring and flower soon thereafter (Chouard 1960). The word "vernalization" is a term used to describe a chilling process used to make the seeds of winter cereals behave like spring cereals (Nils Roll-Hansen 1985). Lysenko's observations on vernalization drew wide attention due to its practical consequences for Russian agriculture. Severe cold and lack of winter snow had destroyed many early winter wheat seedlings. By treating wheat seeds with moisture as well as cold, Lysenko induced them to bear a crop when planted in spring (Michaels, *et al* 2003). Later however, Lysenko inaccurately asserted that the vernalized state could be inherited - i.e., that the offspring of a vernalized plant would behave as if they themselves had also been vernalized and would not require vernalization in order to flower quickly (Amasino, 2004).

Keeping in view this background the present study is an attempt to study the effect of cold temperature coupled with cyanobacterial addition on seed germination and growth of three very important crop plants grown widely in India.

Materials and Methods

In the present investigation Gram *(Cicer arietinum)*, rice *(Oryza sativa)*, and wheat *(Triticum aestivum)* seeds were put in Petri plate to investigate the effect of low temperatures (4, 10,& 25^{o}C) together with cyanobacterial cultures on germination of seeds under laboratory conditions for 5 days. This was followed by potting of the seedlings produced for further studies on vegetative growth of the plants.

5 seeds were added per autoclaved petri dish and 2 mL autoclaved double distilled water (DDW) was added to soak the seeds in control. Similarly in other petri dishes instead of water (DDW) 2 mL of liquid culture of 3 strong nitrogen fixers i.e. *Aulosira, Scytonema, & Haplosiphon* were added to the seeds in the Petri dishes. These 3 algae were a part of the algal culture collection of Laboratory of Algal Biotechnology, Department of Bioscience, Barkatullah University, Bhopal and were grown and maintained by standard microbiological method. Different cyanobacteria were found to influence the germination of different crops in different ways. Best results were obtained with *Aulosira* for gram, *Scytonema* for rice, and *Hapalosiphon* for wheat. So for further experiments, these three specific cyanobacteria were used for the specific crops they produced best results with. The seeds were allowed to germinate for 5 days and all experiments were conducted in triplicates. As the best germination was obtained at 10^{o}C and on cyanobacteria these cold temperature treated seedling were used for pot experiments. For a comparative study the seedlings which were grown at 25^{o}C in laboratory conditions which was also the ambient atmospheric temperature at that time were also potted to further study the effect of cold temperature on the process of germination and vegetative growth of the plants.

Soil used in the pots was sandy loam in texture. 5 Kg dry autoclaved and pulverized soil was filled in each pot and 5 seedlings /pot were planted. Prior transfer to the pots initial growth parameters were recorded at day 5 (Table 2).The pots were maintained in a green house and watered regularly as required. All experiments were conducted in triplicates and results expressed as Mean ± Standard Deviation.

Result and Discussion

Key observations of this study points to the increased germination at 10 º C compared to 25 º C and further increase in vegetative growth parameters in all the 3 crops after transplantation into pots. Cyanobacterial addition further enhanced the stimulatory growth effect. Our results are similar to Nanda *et al*. (1991) showed that, pre-soaking

of pumpkin and cucumber seeds in *Westiellopsis prolific* extract can accelerate seed germination and spraying extracts of this cyanobacterium to emerged seedling during their subsequent cultivation led to significant increase in growth and development of both crops. Table 1 compares the germination of the three crops at day 3 as observed visually. 10 º C was found to support maximum germination in all 3 crops. Table 2 shows the initial growth parameters of the three crops in control (without cyanobacterial addition) and with cyanobacteria grown at 10 ºC at day 5 before potting. Even at the initial stages, addition of cyanobacteria produced better germination and growth compared to control. Table 3 shows the growth parameters of the 3 crops after 45 days of growth in pots. A very distinct difference is clearly evident in the seeds treated with cyanobacterial culture compared to control. Comparison of initial and final results of 10 ºC pretreated seeds showed great stimulation in all the vegetative parameters (Table 4). A significant observation was that cyanobacterial addition with low temperature pretreatment greatly enhanced the growth rate of plants when potted when compared to the controls (Table 5).The percent increase in growth parameters with cyanobacterial addition after 45 days in Gram showed 76% increase in shoot length, 34% in root length, 66% in number of leaves, and 12.5% and 18.4% in Chlorophyll a and b respectively. The increase for Rice was 80%,40.7%,25%,15%,40% for shoot length root length number of leaves and Chlorophyll a and b. For wheat these increases for shoot and root length, number of leaves and Chlorophyll a and b were 25%,42% 14.2% 2.6% and 15.8%.

Table 1: Effect of temperature on the germination measured as shoot length at day 3.

Temperature	Gram	Wheat	Rice
4 º C	+	+	+
10 º C	++++	+++	++++
25 º C	++	+	++

Very poor growth; ++- poor growth; +++-good growth; ++++- very good growth

Table 2: Initial Growth Parameters of Gram, Rice and Wheat grown at 10°C and with or without cyanobacterial addition before transferring to pots on day 5. Cyanobacterial sps indicated here are the ones which gave the best result for that crop. Control here are seeds grown at 10°C in DDW.

Crops	*Shoot length	*Root Length	**Chlorophyll *a*	**Chlorophyll *b*	No. of leaves
Gram					
Control	0.8±0.02	0.12±0.01	2.16±0.13	1.02±0.09	1.52±0.11
Aulosira	1.2±0.03	0.15±0.03	2.37±0.12	1.12±0.17	1.60±0.14
Rice					
Control	0.5±0.009	0.099±0.002	1.76±0.2	0.88±0.03	1.1±0.04
Scytonema	0.8±0.01	0.10±0.01	1.81±0.17	0.94±0.05	1.3±0.02
Wheat	0.8±0.02				
Control	0.95±0.01	0.097±0.003	1.91±0.08	0.96±0.02	0.85±0.028
Hapalosiphon		0.11±0.002	1.96±0.06	1.01±0.09	0.89±0.019

All result are mean±standard deviation (n=3). *-Length in Centimeters. **-µg Chl *a*&*b*/mL.

Table 3: Final Growth Parameters of Gram, Rice and Wheat after potting on day 45.

Crops	*Shoot length	*Root Length	**Chlorophyll *a*	**Chlorophyll *b*	No. of leaves
Gram					
Control	14.2±1.1	3.2±0.4	40.89±2.9	26.76±1.6	15±1.9
Aulosira	25.0±2.7	4.3±0.18	45.56±3.6	30.7±1.5	24±2.1
Rice					
Control	15.0±1.6	2.7±0.09	40.38±2.2	25.3±2.3	8±1.1
Scytonema	27.0±2.9	3.8±0.19	46.70±3.4	35.5±3.1	10±1.7
Wheat					
Control	16.2±1.3	2.6±0.14	39.33±1.9	24.13±2.2	7±1.1
Hapalosiphon	20.0±2.05	3.7±0.17	40.67±3.2	38.17±2.3	8±1.4

All result are mean±standard deviation (n=3). *-Length in Centimeters. **-µg Chl *a*&*b*/mL

Table 4: Fold increase in growth parameters at 45 days compared to initial values taken at 5 days in the 3 crops.

Experimental sets	*Shoot length	*Root Length	**Chlorophyll *a*	**Chlorophyll *b*	No. of leaves
Gram					
Control	15.77	26.66	18.5	25.49	9.8
+ *Aulosira*	20.83	30.7	20.02	28.18	15.6
Rice					
Control	30.0	27.27	22.72	29.27	7.2
+ *Scytonema*	37.0	38.0	28.24	37.23	7.6
Wheat					
Control	20.25	26.8	19.50	26.04	8.2
+*Hapalosiphon*	25.22	33.63	24.33	38.61	8.8

*-Length in Centimeters. **-µg Chl *a*&*b*/mL

Table 5: Percent increase in growth parameters in the 3 crops with addition of cyanobacterial species compared to control at 45 days.

Crops	Shoot length	Root length	Chlorophyll *a*	Chlorophyll *b*	No. of leaves
Gram	78	34.3	20.5	18.4	66
Rice	80	40.7	25.0	40.0	25
Wheat	14	35	42.3	15.8	16.5

When the 10ºC and the 25ºC pretreated plants without cyanobacterial addition were compared the fold increases showed that there was a great variation in the results in low temperature compared to higher temperatures. In Gram plants 1.4,1.39,1.44,1.45, and 1.5 fold increase was obtained in low temperature(10 ºC) compared to higher temperatures (25 ºC) for shoot length, root length, Chl a, Chl b and number of leaves respectively which is a very important finding (Table 6). Similar results were obtained with Rice and Wheat clearly indicating the importance of low temperature pretreatment in the germination process. Currently, we do not know any details of how the duration of cold is measured. What is the cold sensor? In cold-sensing neurons, the cold sensors are cold-responsive calcium channels that transduce the cold signal via altered calcium flux (Story et al 2003).

Table 6: Fold increase in growth parameters of plants given cold treatment of 10 ºC initially for 5 days compared to 25º C in the 3 crops at 45 days.

Crops	Shoot length	Root length	Chlorophyll *a*	Chlorophyll *b*	No. of leaves
Gram	1.4	1.39	1.44	1.45	1.5
Rice	1.35	1.68	1.36	1.54	2.0
Wheat	1.41	1.79	1.5	1.35	1.75

The classic models of vernalization postulate that during prolonged cold, the levels of some factor(s) slowly decline or increase until a threshold is reached that is then transduced into the acquisition of competence to break dormancy of seed/flower. In such a threshold model, the cold sensor could simply be an enzyme that is more or less active than a balancing enzymatic activity in the cold. Also it has been well documented that Gibberellins promotes seed germination in many plant species. Light is a critical environmental determinant for seed germination. Temperature is another crucial external cue that controls seed germination (Bewley and Black, 1982). Although such cold treatment is widely used to improve the frequency and synchronization of germination, the molecular mechanism

underlying this thermoregulation remains unclear. Because of its positive role in seed germination, the effect of cold treatment on GA content previously has been examined in *Pyrus malus* (apple), *Corylus avellana* (hazel), and *A. thaliana* seeds (Ross and Bradbeer, 1971; Sińska *et al.*, 1973) These studies indicate that bioactive GAs were more abundant in cold-treated seeds than in non-cold-treated samples.

However, it is not clear from these initial studies whether low temperature acted as a signal to modulate the GA metabolism. Path way study has demonstrated that GA biosynthesis is activated by low temperature. On one hand cyanobacteria provide the much needed nitrogen and phosphorus and growth stimulators by biosynthesizing growth promoting substances such as auxins, amino acids, sugars and vitamins (Vitamin B12, Folic acid, Nicotinic acid and Pantothenic acid) and on the other hand low temperature also helps production of GA. So the pronounced effect we observe in these experiments is the cumulative effect of low temperature plus effect of cyanobacteria. The other reason that can be suggested for increased plant growth by using cyanobacterial extract is that, the growth of BGA in soil seems to influence the physical and chemical properties of soil. The water stable aggregate significantly increase as a result of algal growth and thereby improves the physical environment of the plants.

The innovation in this study is that, along with vernalization, cyanobacteria which are known to be strong nitrogen fixers and inorganic phosphate providers have been used in the germination process as a medium. Practical applications derived from this study suggests that if farmers soak seeds in cyanobacterial culture or algal samples directly from nature such as nontoxic bloom/algal scum from water bodies to make it economic and germinate the seeds with more vigor, less dormancy period and enhanced agronomic traits it will benefit the crop productivity. If this is combined with chilling temperature treatments a further enhancement can be expected for the crops. Cyanobacteria can be directly added to the nursery beds used to produce seedlings for transplantation. Although further studies are needed on other crops, this technology is a viable ecofriendly process for improving crop cycle of important plants.

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