STUDY ON THE PROMOTION OF LOW TEMPERATURE AND PEG PRETREATMENT ON ELACAGNUS MOLLIS DIELS SEED GERMINATION

L.H.YUAN
College of Life Science, Shanxi Normal University, Linfen, Shanxi 041004, China

ABSTRACT :*Effect of different PEG concentration and soaking seed temperature on activity and germination of seed was studied while Elacagnus mollis Diels seeds were the material. Results indicated under 25% PEG and 2℃, osmotic adjustment was good, and vigor index was increased significantly; germination rate was significantly increased compare to control group; seed quality was improve; plant resistance was strengthened. Analysis of physiological and biochemical indexes indicated that content of soluble protein and heat-stable protein and activity of SOD and CAT in seed increased under this condition. However, MDA content and POD activity decreased.*

Keywords:Elacagnus mollis seed, low temperature stress, PEG, pretreatment

1. Introduction

Elaeagnus mollis Diels, of Elaeagnus, Elaeagnaceae, is a national two-tier protection of rare and endangered plants, Chinese endemic species, mainly distributed in the Yellow River basin and Shaanxi Weihe basin. This superior tree species can afforest barren mountains, improve soil conditions and prevent soil erosion. While some factors, such as special structure of fruit, short life of seed, lack of competitiveness of Elaeagnus mollis seedlings and serious damage from humans' activities have caused its endangerment[1]. Numerous researches indicated that low temperature pretreatment could enhance seed germination rate, germination index, activity, resistance and so on [2],[3]. And PEG pretreatment could promote seed germination, shorten emerge time, as well as enhance seed activity and resistance [4],[5],[6]. In this research, low temperature and different mass fraction of PEG were used to treat seeds to measure change of their relevant physiological and biochemical indexes in order to provide theory basis for improving the sowing work of this endangered plant.

2.Materials and methods

2.1 Materials

Seeds were collected from Ganquan forest farm in Yicheng country in the autumn of 2007. After collecting, epicarp and mesocarp were abandoned; and then seeds were air-dry and storage [7]. In the spring of 2014, seeds were used to experiment in greenhouse in College of Life Science in Shanxi Normal University. PEG (6000) was sub package products imported from Japan.

2.2 Methods

2.2.1 PEG pretreatment Refer to method of Bingchu Song *et al.* [6]

Seeds were distributed to different culture dishes under room temperature with 21℃, and then PEG with mass fraction 1%, 5%, 15%, 25% and 35% were added in order. Seeds were completely soaked in solution. Deionized water was as the control group. Total soaking time was 24h. Culture dishes should be shaken for every 3h to good ventilation. Each treatment has 3 repeats.

2.2.2 Low temperature stress treatment

After above pretreatment, seeds were washed by distilled water till cleaning the drugs. Then wet seeds were put back to test tube and immediately put into O~2℃ refrigerator for 24h to low temperature stress treatment.

2.2.3 Germination experiment

After low temperature stress treatment, 50 seeds were taken from each treatment (pot routine management). Seeds stayed in germinating box(temperature was (21±2) ℃, illumination was 2000 Ix, illumination for 24h) for 10 days to germinate. Germination rate, germination index and activity index of seeds were measured. Thereinto,

germination index Gt=ΣGt/Dt (Gt is the number of germination at t days, Dt is relevant days of germination); activity index Vi=SΣ Gt/Dt (S is the average length of seedling).

2.2.4 Water absorption curve measurement

During PFG pretreatment of seeds, 10 seeds were taken to measure water absorption curve at 0, 1, 3, 5, 7 and 9h, respectively after treatment.

2.2.5 Protein content measurement

Coomassie brilliant blue staining was taken [8]. 0.1g plant material was grinded and abstracted in 0.5ml 50mmol/L pH6.3 phosphate buffer. After 10 min centrifugation at 15000 rpm, supernatant was taken to measure soluble protein first, and then boiling-stable protein was measured.

2.2.6 MDA content measurement

Refer to the method of Shijie Zhao *et al.*[9].

2.2.7 SOD activity measurement

NBT photochemistry reduction method was taken [8].

2.2.8 POD activity measurement

Callus wood phenol method was taken [8].

2.2.9 CAT activity measurement

Permanganate titration was taken[8].

3. Results and discussions

3.1 Effect of low temperature and PEG pretreatment on seed germination

According to Table 1, for PEG pretreatment followed by low temperature treatment, 1%, 15%, 25％and 35％ PEG all can increase seeds germination. And 15% and 25% are the best. 15%, 25％and 35％ PEG not only increase seedling length(sum of radical and hypocotyls), but increase seedling germination index and significantly increase activity index of seed. It indicates PEG pretreatment with mass fraction 15%, 25％and 35％ all have good influence on Elacagnus mollis Diels seed germination. In general, 25% PEG has the best effect.

Table 1 Influence on seed germination of low temperature and PEG preprocess

PEG /%	Germi n-ation rate /%	Height of seedlings /cm	Germin a-tion index	Vigor index
Cont -rast	63.3	1.89	4.65	9.1668
1	64.8	1.92	4.79	9.1296
5	68.4	1.97	5.12	10.1224
15	81.6	3.26	12.25	40.118**
25	83.7	3.27	13.01	42.5325**
35	70.5	2.96	7.25	21.4963**

Note：* Stand for significant corrolelation at a=0.05;
**Stand for more significant correlalion at a=0.01.

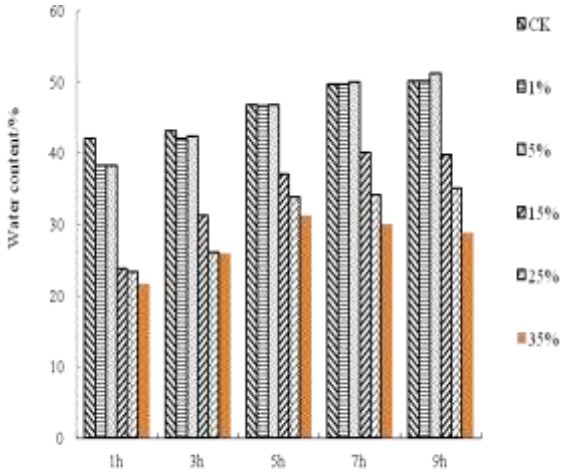

Fig.1 Effect of PEG pretreatment on water content in Photinia seeds during the first

3.2 Effect of low temperature and PEG pretreatment seed water absorption rate

Fig.1 indicates 1% and 5% PEG have no significant influence on seed water absorption rate. But 15％, 25％ and 35％ PEG can significantly reduce seed water absorption rate. And this effect becomes stronger with PEG mass fraction increasing. Dry seed cell contains a lot of big molecular substances which will reduce seed matric potential to lower seed water potential [10],[11]. Water potential of dry seed is about -20.0Mpa. When it is put into water with 0 water potential, water will go into seed quickly. But in PEG solution, because PEG a macromolecule osmotic agent which can decrease osmotic potential of solution to decrease water potential of

solution, seed water absorption rate can be reduced by reduce the water potential difference between internal of seed and solution. This tendency will be more obvious with PEG mass fraction increasing [12].

Table 2 Biochemical index of seeds with 25% PEG and low temperature stress treatment

Item	Contrast	Treatment
Soluble protein / (ug·g-1)	489.6	1369.8
Heat stable protein / (ug·g-1)	298.3	400.1
MDA / (umol·g-1)	11.23	9.21
SOD / (unit·g-1)	76.3	174.2
POD / (unit·g-1)	392.1	180.2
CAT / (unit·g-1)	1.96	11.3

3.3 Effect of low temperature and PEG pretreatment on seed protein content and resistance

Table 2 shows that soluble protein content of seed with 25% PEG and low temperature treatment increases by 180% over control group. It represents that soluble protein content of seed with PEG pretreatment increases though it was been through a long term(24h) of low temperature stress. To some extent, soluble protein content of plant after low temperature stress represents the content of all kinds of enzymes and other big molecule proteins in plant cell [13]. For the seed which is just germination, content of soluble protein reflects the metabolic activity of seed to some degree. This result from a side indicates that low temperature and PEG treatment can increase the resistance of seed in germination. Table 2 also indicates boiling stable proteins content of seed with 25% PEG and low temperature stress treatment increases by 34.12% over control group. Boiling proteins are always the induced products of cold-acclimation, ABA or dehydration, etc. This kind of protein has a significant property which is thermal stability (which means it can still be in the solution after 10min boiling). They have high hydrophilic and specific amino acid sequence, and can regulate osmotic potential. It can be inferred that it has stationary film and antifreeze [14],[15]. The result shows the changing of boiling stable protein is similar to soluble protein. It means resistance of seed in germination can be enhanced with the increasing of soluble proteins and boiling stable proteins.

3.4 Effect on membrane lipid peroxidation

According to table 2, after seeds with PEG pretreatment is treated with low temperature stress, activity of SOD and CAT significantly increase compare to control group, specifically increasing 128% and 476%, respectively. MDA content and POD activity are lower than control group, specifically decreasing 17.99% and 54.04%. Main function of SOD is catalyzing superoxide anion free radical to disproportionate reaction which produces H_20_2 & O_2 to eliminate injure to plant from free radical. During above results, SOD activity of seed with PEG and low temperature treatment significantly increases. It indicates SOD activity of control group seeds may be under inhibitory state influenced by low temperature stress. However, low temperature and PEG pretreatment can reduce this kind of inhibition. Though SOD can clear free radical, H_20_2 will be generated. As an oxidant, H_20_2 can be harm to plant. It can produce hydroxyl radical and Singlet molecular oxygen which have great oxidizability. Accumulation of excess H_20_2 will be great harm to plant. High concentration of H_20_2 in plant tissue is mainly removed by CAT to control it at a lower level [16]. Effect of POD on plant is quite complex. For now, it is regarded as an enzyme with double effects. For one hand, it has protective effect. The reaction principle is POD can catalyze the reaction between relevant substrate (like phenolic compound) and H_20_2 to eliminate H_20_2. On the other hand, POD participates in the degradation of chlorophyll and production of active oxygen and trigger membrane lipid peroxidation which is the product of plant senescence to certain stage. It is an injury reaction and can be the index of plant senescence [17]. Base on the effect of POD on Elacagnus mollis Diels seed in this experiment, it can be inferred that is a protective reaction. The reason of its low activity may be the CAT activity of pretreatment seeds is high which can clear numerous H_20_2. And the activation of POD may need a process. And it may need a certain condition (for example a certain amount of H_20_2) to realize.

MDA is the final product of membrane lipid peroxidation. It has been proved that accumulation of MDA was resulted from degradation of unsaturated fatty acid [18],[19] and was caused by free radical [20]. To some extent, therefore, accumulation of MDA reflects the activity status of internal free radicals. Large

accumulation indicates high level of superoxide anion and hydroxyl radical.

4.Acknowledgements

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