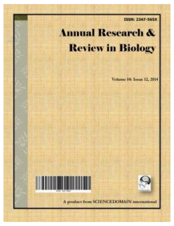

Annual Research & Review in Biology

29(3): 1-7, 2018; Article no.ARRB.44852
ISSN: 2347-565X, NLM ID: 101632869

Temperature Effect on Traditional Pear (*Pyrus communis* L.) Landraces Seed Germination

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Authors' contributions

This work was carried out in collaboration among the authors. Author SJ designed the study, carried out the laboratory research, contributed with the applied laboratory methodology for the method and temperatures of seed stratification according to the previous research, analyzed the obtained results, literature searches and manuscript writing and is corresponded author respectively. Author EMM performed the statistical analysis of data, manuscript writing and complemented to the literature searches. Both authors read and approved the final manuscript.

Article Information

DOI: 10.9734/ARRB/2018/44852
Editor(s):
(1) Dr. George Perry, Dean and Professor of Biology, University of Texas at San Antonio, USA.
Reviewers:
(1) R. Mahalakshmi, India.
(2) Ezekiel Esho, University of Ibadan, Nigeria.
(3) Tiga Neya, Kwame Nkrumah University of Science and Technology, Ghana.
Complete Peer review History: http://www.sciencedomain.org/review-history/27045

Original Research Article

Received 12 August 2018
Accepted 29 October 2018
Published 05 November 2018

ABSTRACT

Investigations included six traditional pear landraces (*Pyrus communis* L.): Aleksandra, Vodenka, Patlidjanka, Ljubichica, Tatlikuti and Crvenushk*a*. The number of seeds per fruit, seed energy and total seed germination were investigated and statistically analysed (P = .05 and P = .01). Seed stratification encompassed pre-chilling by using three treatments: I treatment covers seed pre-chilling at -18°C for 24 hours; II treatment covers seed pre-chilling at +1°C for 2 weeks; III treatment covers seed pre-chilling at +8°C for 1 week. Only Patlidjanka belongs to the group with high seed number in the fruit. The other landraces produced low seed number like 2-3 seeds per fruit. The average number of all researched landraces is 3.67 seeds per fruit. The pear Tatlikuti showed a very high significant difference (P = .01). It was concluded that III treatment showed the highest seed energy at landraces Ljubichica 77.5% and Tatlikuti 75.0% and the lowest at Aleksandra 52.5%. There were significant differences (P = .05) between the applied treatments regarding seed energy.

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In II treatment, the total seed germination had the highest effect on Patlidjanka 87.75%. The values for germination seeds in the other landraces were in the range of 77.5% to 86.25%. Statistically, a significant difference was determined in the landraces Ljubichica and Crvenushka (P = .05), and there was high significant difference (P = .01) in the pear Aleksandra. The II and III treatments could be recommended as the most suitable to improve the pear seed germination.

Keywords: Pear; Pyrus communis L.; traditional landraces; seed stratification; seed number per fruit; total germination.

1. INTRODUCTION

The best way to preserve the purity of seed - producing plant genotype is reproduction directly from the seed. The obtained seedlings can further be used to raise rootstocks. In the case of fruit crops, raising the seedlings from the seed is very specific as mainly fruit crop seeds germinate poorly [1]. The conditions of the maternal (thermal) environment and the seed dormancy are in balance and in nature, this is evolutionarily synchronised. Therefore, in laboratory, should be enable temperature conditions which as the main environmental signal would influence the seed's dormancy cycle [2]. Therefore, in fruit plants, in most cases, a vegetative way of propagation is practised (grafting, shield budding, layering) and is quite successful [3-5]. But, in this way, the genetic purity is modified and does not correspond with the "Second GPA" (Global Plan Action) that directly addresses the opportunities, developments and challenges concerning joy plant conservation and use in the 21st century [6].

In natural conditions, the dormancy of seeds of fruit plants that are hydrated is at least 3 weeks, and on average ranges from 2-5 weeks (in some cases even longer) [7,8]. For these reasons, various ways of seed dormancy breaking are applied: light and alternating temperature reduce the seed dormancy leading do degradation of abscisic acid (ABA), pre-chilling (stratification) may induce leakage of ABA, scarification, hydration and germination in the presence of KNO_3 or GA3, or adding growth regulators can break dormancy [9]. Stratification is so called “moist-chilling” that breaks down physiological and biochemical dormancy [10]. This process involves "seed hydrating" by adding water, adsorption of water, followed by moist-chilling [11]. The intake of water inside the seeds breaks seed dormancy and allows to activate the physiological processes that activate the embryo [12]. In the same vein, seed soaking contributes to the softening of the seed coat (testa) so the embryo starts to elongate and the appearance of the seedling and the newly formed root (radicle) appearance becomes observable [13]. This way is completed the process of seed germination [14]. So far, no generally accepted method has been found to improve seed germination. It is commonly known that the capacity of the seed for germination can be improved and increased, but only when the seeds are assumed to be viable [15]. Plant hormones such as abscisic acid (ABA), gibberellins, auxins (IAA), cytokines and ethylene as well as biochemical substances have a role in control of many physiological and biochemical processes in the plant [16,17]. Seed dormancy can be interrupted by gibberelic acid (GA) and other several factors like temperature, light, radiation and ultrasound [9,18].

In pears, the seed coat (testa) is fairly solid and impregnated with waxes and fats, which makes even more resistance. The seed reserve substances are placed inside the major seed storage organ (endosperm) and their chemical composition is different and it is a variable component, unlike the seed testa which is genetically determined [19] and varies depending on the landraces and pear varieties [20]. Only the chemical composition can be modified to a certain extent depending on the agronomic practice. Kratovalieva et al. [1] pointed out that seed germination ability does not depend of genotype and could be managed and improved through appropriate seed treatments. Because of a deficit of seed testing investigations, this was considered as a challenge for the research team.

Taking into account the significance of the investigated traditional pear landraces [21,22,23,24,25,26], as well as the interest of the local inhabitants for their preservation from permanent loss, we did this investigation for improving the way of generative production of pear. The main goal was to get seedlings from the seed to preserve the original genotype and their autochthonous.

2. MATERIALS AND METHODS

2.1 Plant Material

Investigations that were carried out encompassed six traditional pear landraces (*Pyrus communis* L.). According to the time of maturation are divided into summer (Aleksandra), autumn (Vodenka, Patlidjanka, Ljubichica and Tatlikuti) and winter landraces (Crvenushka). The landrace Vodenka has been chosen as standard in statistical analysis. Pears originate from the territory of Macedonia and are mainly individual trees. For examinations, it was important to have 40 seeds of each landraces in order to be set for germination in 4 replications, but not the number of pear fruits.

2.2 Seed Preparation for Germination

The pear fruit seeds were removed manually and the number of seeds in the fruit was counted. The removed seeds were left on a filter paper to dry at room temperature 20-25°C for a 5 day period. The process of preparation and placement of seeds on the germination substrate implies thorough seed washing and 12 hours water soaking prior to setting up for germination. Each treatment was carried out with 40 seeds of each landrace radial and evenly distributed on filter paper at +18°C in four replications (10 x 4) i.e. a total of 40 healthy, well-developed and formed seeds were used for each landrace. Every day they were aerated for 5 minutes and supplied with moisture using manual sprayer. Germination period was prolonged in order to examine the best conditions for seed germination, but after 18 days first sprouts appeared and grew until 30 days. The seeds' energy was determined on the 20th day after the seeds were set up by counting those seedlings occurred as normally developed with cotyledons, healthy and free of pathogens. In the same way by counting, the total germination on the 30th day is also determined when there are two true leaves on the seedlings.

2.3 Method of Seed Pre-chilling

Pear seeds have a hard coat, thereby must be stratified they are subjected to a certain period of pre-chilling by storage in the freezer. In this context there were carried out three stratification treatments: I treatment covers seed pre-chilling in refrigerator at -18°C for 24 hours; II treatment covers seed pre-chilling in refrigerator at +1°C for 2 weeks; III treatment covers seed pre-chilling in refrigerator at +8°C for 1 week. After removal from the freezer seed packages were left on adaptation at room temperature for 24 hours without unpacking the seeds. Afterwards, with all treatments, seeds were placed in a container and covered with water during 12 hours to perform seed imbibitions. For the first treatment seeds were packed in a double water resistant paper bag which were placed in double plastic bags. This way of putting the seeds is applied in order to avoid the freezing of the free water found in the seeds and from which the formation of crystals may cause mechanical damage to the cells.

2.4 Statistical Analysis of Data

The results of the survey on the possible benefits of the conducted pre-chilling treatments for a higher potential of seed germination in four replications were subjected to statistical analysis. With the statistical analysis, a significant difference was found on the two levels $P = .05$ and $P = .01$. Obtained results for analysed traits were processed by analysis of variance (ANOVA) and LSD test.

3. RESULTS AND DISCUSSION

3.1 Number of Seeds per Fruit

The average number of seeds per fruit in all researched landraces is 3.67 seeds. The landrace Patlidjanka has the largest number of seeds per fruit 5.2 (Table 1). It should be emphasised that Aleksandra (4.30) and Tatlikuti (4.20) also have a large average number of seeds per fruit. The landraces Vodenka (2.9), Ljubichica (2.50) and Crvenushka (2.90) have a small number of seeds. For this characteristic, the pear Tatlikuti showed a very significant difference ($P = .01$) compared to the standard landrace Vodenka (Table 2).

Table 1. Mean values of seed number per pear fruit in investigated landraces (x=10 ± StDev)

Landraces					
Vodenka	**Aleksandra**	**Patlidjanka**	**Ljubichica**	**Tatlikuti**	**Crvenushka**
2.90 ± 0.57	4.30±0.67	5.20±0.79	2.50±0.53	4.20**±0.63	2.90±0.88
All landraces	3.67±0.19				
LSD0.05	0.62*				
LSD0.01	0.83**				

** The mean difference is significant at the 0.05 level.*
*** The mean difference is significant at the 0.01 level*

Table 2. Analysis of variance of the seed number per pear fruit

ANOVA						
Source of variation	***SS***	***df***	***MS***	***F***	***P-value***	***F crit***
Between Groups	55,73333	5	11,14667	23,5125	1,83E-12	2,38607
Within Groups	25,6	54	0,474074			
Total	81,33333	59				

3.2 Assessment of Seed Energy and Seed Germination

3.2.1 Seed energy assessment

In terms of seed energy in the I treatment there is a weak interaction between seeds and treatment, so there is evident a very low seed energy. Namely, the lowest seed energy is found in the pear Patlidjanka (30.0%), while in the landrace Ljubichica is the highest (40.0%) (Table 3). No significant differences were noted between pear landraces statisticaly. According to Bewley and Black [27], the seeds respond well and biochemical-physiological processes are activated in the seeds treatments carried out to break seed dormancy. In II treatment, the highest energy of seed germination shows the landrace Tatlikuti (65.0%), and the lowest in the pear Crvenushka (47.5%). This landrace (Crvenushka) showed a very significant statistical difference in relation to the remaining landraces (*P* = .01) (Table 4). The III treatment increased higher seed energy in all landraces except in Aleksandra. In the landrace Vodenka II and III treatments had the same effect regarding seed energy. It was concluded that in III treatment the highest seed energy was expressed in landraces Ljubichica (77.5%) and Tatlikuti (75.0%) and the lowest value was noticed in the pear Aleksandra (52.5%). Statistically significant difference was found in the landrace Tatlikuti (*P* = .05) and high significant difference in the pear Ljubichica (*P* = .01). Also, for this characteristic, high significant differences (*P* = .01) was detected statistically between applied treatments.

3.2.2 Seed germination

In the I treatment, seed germination has the lowest value in the landrace Vodenka (54.2%), while the highest value in the pear Tatlikuti (62.5%). In the pear Ljubichica, Tatlikuti and Crvenushka were found to have high significant differences (Table 3). The second treatment had the highest effect in the landrace Patlidjanka (87.75%), as well as in all other landraces caused a similar high germination rates ranging from 77.50% to 86.25%. Statistically, significant difference was determined in the pears Ljubichica and Crvenushka (*P* =.05), and high significant difference (*P* = .01) in the pear Aleksandra. Unlike the II treatment that has the strongest impact on the seed germination potential, III treatment has a slightly lower impact but should not be neglected and can be practiced (Fig. 1). In the III treatment, the lowest germination rate was found again in the landrace Alexandra (75.75%), which correspond to the lowest energy. The highest seed germination as well as seed energy was observed in the pear Tatlikuti (83.5%), which statistically showed a high significant difference (*P* = .01) as well as in Ljubichica. The statistical analysis between treatments showed that there is a very strong significance differences between I and II treatments and between I and III (*P* = .01), while between II and III the difference is significant (*P* = .05) (Table 4).

Table 3. Mean value of seed energy and seed germination in pear fruit (%) (x=10 ± StDev)

Landraces	**Treatments**					
	Seed energy			**Seed germination**		
	I	**II**	**III**	**I**	**II**	**III**
Vodenka	35.0±12.91	60.0±8.16	60.0±11.54	54.25±1.71	85.50±1.29	77.50±1.29
Aleksandra	32.5±5.00	55.0±5.77	52.5±5.00	57.25±1.71	77.50**±1.29	75.75±1.71
Patlidjnka	30.0±8.20	50.0±8.16	60.0±8.16	57.25±2.22	87.75±0.96	80.00±3.65
Ljubichica	40.0±8.20	60.0±8.16	77.5**±9.57	60.50**±1.91	82.50*±2.38	82.50**±2.08
Tatlikuti	37.5±9.60	65.0±5.77	75.0*±5.77	62.50**±2.64	86.25±1.71	83.50**±2.38
Crvenushka	35.0±5.80	47.5**±5.00	65.0±5.77	60.75**±2.50	82.25*±1.71	77.25±2.62
LSD0.05	**12.860**	**10.357**	**11.874**	**3.190**	**2.406**	**3.579**
LSD0.01	**17.620**	**14.190**	**16.268**	**4.371**	**3.297**	**4.904**

** The mean difference is significant at the 0.05 level.*
*** The mean difference is significant at the 0.01 level*

Table 4. Analysis of variance of different treatment effect on pear seed germination

ANOVA						
Source of variation	***SS***	***df***	***MS***	***F***	***P-value***	***F crit***
Between Groups	2856.25	2	1428.125	28.68201	7.49E-06	3.68232
Within Groups	746.875	15	49.79167			
Total	3603.125	17				

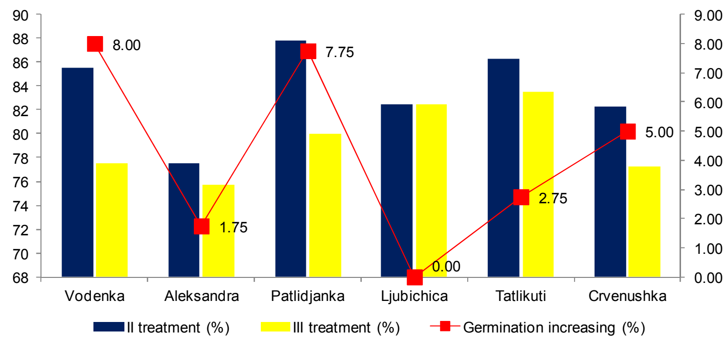

Fig. 1. Increased impact of II treatment as best suited on pear seed germination as the most efficient (%)

Pears are self-incompatible species and most are not self-fertile. Also it is a known fact that pear naturally gives partenocarp fruits [28]. Seeds are rich in enzymes and hormones such as auxins, gibberellins and cytokines, and they influence not only on the development of the fruit, but even on the form. These plant genetic resources (PGR) are interesting for research due to this, but also because of the parthenocarpy (fruit set without fertilisation). Considering that carpel number is varietal trait and is well known pear flowers have 5 stigmas, they can mostly form 10 seeds.

The pear is a diploid plant (2n = 34), but not all investigated landraces. The amount of seeds in the fruits is different and is a genetic feature [28]. Kratovalieva et al. [1] states that studies are the first of this type, but at the same time gave a review of the fertility of the pear genotype and the possible correlation between the number of seeds in the fruit and fertility. Münzbergova and Skuhrovec [29] pointed out on strong link between the number of undamaged seeds and ploidy level.

While in nature there is a natural process of cooling the seeds during the winter months, a simulation is carried out in laboratory conditions in order to find the most suitable method of stratification and interrupting of seed dormancy [30]. Pre-chilling is necessary because interrupts the dormancy (rest) of seed embryo allowing moisture, CO_2 and an appropriate temperature regime as suitable to initiate biochemical processes leading to the preparation of embryo germination. Kratovalieva et al. [1] examined the impact of various stratification treatments on some summer pear landraces and found that a low temperature of -18°C is necessary to be stopped the seed dormancy and induction of biochemical and physiological processes in the endosperm of the seed. Although there are older literature data [31,32,33], however, guided by the seed physiology, even the keeping under the positive low temperatures (+4°C to +6°C) [27] intensify the metabolic processes in the seeds. The implemented treatments differently interrupt the dormancy of the seeds, but each of them causes physical changes that are associated with hydrolytic biochemical processes [28]. With seed imbibitions, metabolic processes are induced. Namely, the seed reserve substances absorb water, stimulates the embryo to produce phytohormones (gibberellins mainly) which pass through the aleuronic layer of the seed coat by sending signals for the synthesis of α-amylase and other hydrolytic enzymes for the decomposition of carbohydrates, lipids and proteins [34].

In fact, with these trials and obtained results, it is possible to shorten the long seed stratification period (30-90 days) with seed pre-chilling in refrigerator at +1°C for 2 weeks (II treatment). The research is of interest and will continue, as it opens the possibility of generic propagation of the pear, obtaining generic substrates for pear grafting on their own rootstock, which is the best way for preserving the purity of the landraces and for maintaining the traditional pear gene pool.

4. CONCLUSION

The average number of seeds per fruit is 3.67 at all researched traditional landraces. The landrace Patlidjanka has the largest number of seeds per fruit 5.2 while landrace Ljubichica produces the smallest number of seeds per fruit in average 2.5. Regarding seed number per fruit, Tatlikuti showed a very high significant difference (P = .01). In the III treatment the highest seed energy was found in the landraces Ljubichica (77.5%) and Tatlikuti (75.0%). Between applied treatments, high significant differences (P = .01) was founded statistically for seed energy. The II treatment, showed the highest effect in the pear Patlidjanka (87.75%). In generally other landraces showed similar high seed germination (77.70% - 86.2%). For seed germination, in II treatment, statistically significant difference was determined in the landraces Ljubichica and Crvenushka (P = .05), and very significant difference (P = .01) in the pear Aleksandra. The II and the III treatments are recommended to improve seed germination.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history/27045