EMERGENCE AND MORTALITY OF SEEDLINGS IN SOME APPLE (*Malus* × *domestica* Borkh.) PROGENIES

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(Received August 8, 2006/ Accepted November 2, 2006)

A B S T R A C T

The experiment was conducted on 58 apple hybrid families in the period 2004-2006. The seeds for germination were prepared by stratification in whole fruits and subsequently by a complementary treatment in a peat substrate in multipots for 43 to 92 days. Seedling emergences were on average over 95% in each year. The influence of the duration of the complementary stratification or the parent cultivar on the percentage of seedlings that emerged was generally small. Mortality of young seedlings due to the expression of the pale green lethal gene *l* was noted in 10 populations. Cultivars which turned out to be heterozygotes *Ll* were 'Arlet', 'Delbarestivale', 'Fuji', 'Ligol', 'Redfree', 'Rubinola', and 'Šampion', whereas 'Beauty of Repty', 'Bolero', 'Braeburn', 'Gala Must', 'Granny Smith', 'Kosztela', 'Lobo', 'McIntosh Wijcik', 'Otava', 'Priscilla', 'Redspur', 'Rubin', 'Spartan 2/8', and 'Topaz' appeared to be homozygotes *LL*.

Key words: apple, stratification, seedling emergence, pale green lethal gene *l*.

INTRODUCTION

Production of hybrid seedlings, which follows crossings, is part of the breeding process. Literature data points to some variability in seed germination and seedling emergence among apple progenies, and the methods of after-ripening seed treatments (Keulemans et al., 1994; Pitera, 2003; Pitera and Odziemkowski, 2003; Pitera et al., 2004).

Traditional stratification is commonly used to break apple seed dormancy and usually gives satisfactory results (Trancred et al., 1995).

Stratification may be simplified by firstly storing fruits from controlled crosses, then extracting seeds, placing them in a peat substrate and holding them under optimum growth conditions (Janick et al., 1996). However, such a procedure often results in a quite low proportion of seedlings vs. the number of seeds sown (Pitera and Odziemkowski, 2003). A new method of stratification has been recently developed by Pitera and Odziemkowski (Pitera, 2003; Pitera et al., 2004). It is advisable to use this method in apple breeding as well as in the production of the Caucasian pear seedling rootstock (Odziemkowski et al., 2004). We applied this new method to examine seedling emergence in various crossbred apple progenies.

The genetics of apple (*Malus*) is very complex due to a high degree of heterozygosity of that genus (Janick et al., 1996). Numerous genes govern many important tree and fruit features. Therefore, an effective selection is possible within large segregating apple progenies (Alston, 1987; White, 2000). Some traits, however, are under the control of single genes in heterozygous or homozygous (dominant or recessive) conditions. Thus, it is essential for the apple breeder to be familiar with a complete list of all the known genes of *Malus* (Alston et al., 2000).

Expression of some genes results in the mortality of young apple seedlings (Tydemann, 1933; Klein et al., 1961; Alston, 1976; Way et al., 1976; Gao and van de Weg, 2006). The recessive gene *l* (pale green lethal) causes a chlorophyll deficiency in seedlings, which appears shortly after germination and subsequently leads to their death at the cotyledon stage. Identification of such genotypes of cultivated apple varieties has been the subject of a few studies (Klein et al., 1961; Alston, 1976; Way et al., 1976) as well as of our own investigation described in this paper.

MATERIAL AND METHODS

Controlled crosses were made in 2003, 2004 and 2005 between 48 apple (*Malus* × *domestica* Borkh.) cultivars. The numbers of crosses involving the individual cultivars were as follows: 32 – 'Ligol'; 8 – 'Rubin'; 7 – 'Topaz'; 4 – 'Braeburn', 'Fiesta', 'Idared'; 3 – 'Delbarestivale', 'Lobo'; 2 – 'Fuji', 'Gala', 'Gala Must', 'Gloster', 'Granny Smith', 'Kosztela', 'Priscilla', 'Redspur', 'Rubinola', 'Šampion', 'U 641'; 1 – 'Alwa', 'Arlet', 'Bancroft', 'Beauty of Repty', 'Bolero', 'Cox's Orange Pippin', 'Delbard Jubilé', 'Elstar', 'Florina', 'Golden Delicious', 'King of the Pippins', 'Ligolina', 'Linda', 'Maigold', 'McIntosh Wijcik', 'Odra', 'Otava', 'Pinova', 'Priam', 'Prima', 'Primula', 'Redfree', 'Rubinette', 'Spartan', 'Spartan 2/8', 'U 1165', 'Vanda', 'Wealthy' and 'Yellow Transparent'.

The method of seed stratification was adopted from Pitera (2003) and Pitera and Odziemkowski (2003). For this purpose, each year fruits were harvested on nearly the standard picking date according to

the literature which describes apple cultivars grown in Poland, and then stored in a common cool store for 4-6 months. Subsequently, during the periods February 19 – March 19, 2004 and February 17 – March 5, 2005, and on February 6, 2006, seeds were removed from the fruits and sown into QuickPot 96 T trays containing a sterilized peat substrate, and placed in the cool chamber again (for complementary or additional stratification).

In the year 2004, 12660 seeds were studied (91-1970 seeds, depending on the family); 12650 seeds in 2005 (in families of 92-1953 seeds); and 162 seeds in 2006 (84 or 78 seeds/family).

Multipots with the seeds of 2003 were transferred to a plastic tunnel on May 1 or 7, 2004, i.e. 43-72 days after sowing, those of 2004 – on April 28, 2005, i.e. after 54-70 days, and those of 2005 – on May 11, 2006, after 93 days.

Then, after 2-3 weeks, records were made of seedling emergence and the presence of pale green seedlings, which all died within the next 1-2 weeks. The mortality of the seedlings was assumed to be a result of the expression of the pale green lethal gene *l* in the homozygous state (Klein et al., 1961; Alston, 1976; Way et al., 1976). The results concerning the agreement on a 3:1 basis in the progenies segregating for the pale green lethal gene were elaborated with a chi square test at P=0.05.

RESULTS

In 2004, the total percentage of seedling emergence amounted to 95.5%, but in individual populations it varied between 85.2 and 100% (Tab. 1). In 2005, the average seedling emergence rate was 97.8%, with the range of 89.9-100% (Tab. 2), and in 2006 the rate averaged 95.6% (Tab. 3).

Considering the influence of the female parent on seed germination and seedling emergence, the highest percentage of seedlings in 2004 was obtained in the families that included the cultivars ‘Delbarestivale’, ‘Spartan 2/8’, ‘Spartan’, ‘Redspur’ and ‘Priscilla’ (over 98%). Slightly lower emergence (95-98%) was exhibited by the seeds of ‘Florina’, ‘Gloster’, ‘Granny Smith’, ‘Idared’, ‘Ligol’, ‘Ligolina’, ‘Rubinette’, ‘Šampion’ and ‘Wealthy’. However, the lowest seedling production was noted in the families of ‘Fiesta’ and ‘Fuji’ as well as ‘Ligol’ pollinated by columnar apples ‘Bolero’ and ‘McIntosh Wijcik’ (approx. 85-90%) (Tab. 1).

The emergence of seedlings in 2005 was highest in combinations with the cultivars ‘Delbard Jubilé’, ‘Bancroft’, ‘Elstar’, ‘Kosztela’, ‘Golden Delicious’, ‘Rubin’, ‘Priscilla’ and ‘Redspur’ (over 98%). The lowest seedling emergence was shown by the ‘Fiesta’ family (approx. 90%). The seeds of ‘Ligol’ produced on average 97.6% of seedlings, within the range from 95.5 to 98.9%. Families with ‘Rubin’ as the female parent averaged 98.2% (95.8-99.8%) (Tab. 2).

In 2006, the seeds of ‘Alwa’ emerged at a lower rate than those of ‘Maigold’, despite the same pollen parent, cv. ‘Topaz’ (Tab. 3).

Table 1. Selected characteristics of apple progenies in 2004

Progeny	Days of additional stratification at 2-3°C	No. of seeds	Seedling emergence [%]	Pale green lethal seedlings [%]	χ^2 3:1	p
Delbarestivale × Ligol	60	80	100	31.3	1.67	0.2
Fiesta × Gloster	53	1137	85.2			
Fiesta × Ligol	53	1343	85.9			
Florina × Ligol	58	175	97.7			
Fuji × Ligol	49	227	89.4	23.2	0.37	0.55
Gala × Ligol	49	216	94.9			
Gloster × Fiesta	43	114	98.2			
Gloster × Fiesta	49	96	96.9			
Granny Smith × Ligol	43	192	97.7			
Idared × Prima	53	712	97.3			
Cox's Orange Pippin × Delbarestivale	58	96	91.7			
Ligol × Bolero	60	773	87.2			
Ligol × Delbarestivale	43	91	93.4	20.0	1.13	0.3
Ligol × Idared	46	176	96.6			
Ligol × Idared	49	632	97.8			
Ligol × Otava	46	715	95.7			
Ligol × Rubinola	43	193	97.4	17.6	5.56	0.01
Ligol × Rubinola	49	111	97.3	25.0	0.00	1
Ligol × Topaz	46	356	97.5			
Ligol × U 641	72	1970	96.9			
Ligol × McIntosh Wijcik	46	593	90.1			
Ligolina × Topaz	43	143	97.9			
Priscilla × Ligol	49	265	98.1			
Redfree × Ligol	60	154	93.5	23.6	0.15	0.7
Redspur × Rubinola	46	129	98.4			
Rubinette × Idared	43	156	97.4			
Rubinette × Idared	49	193	98.4			
Šampion × Idared	58	421	97.9			
Šampion × Ligol	43	482	99.0	23.1	0.96	0.35
Šampion × Ligol	58	179	91.1	29.4	1.72	0.2
Spartan 2/8 × Ligol	49	144	99.3			
Spartan × Ligol	59	250	99.2			
Wealthy × Yellow Transparent	60	146	97.3			
Total		**12660**				
Average			**95.5**	**24.1**		

Table 2. Selected characteristics of apple progenies in 2005

Progeny	Days of additional stratification at 2-3°C	No. of seeds	Seedling emergence [%]	Pale green lethal seedlings [%]	χ^2 3:1	p
Bankroft × Primula	55	109	100			
Delbard Jubilé × Topaz	55	123	100			
Elstar × Topaz	55	92	98.9			
Fiesta × Braeburn	55	138	89.9			
Gala Must × Braeburn	60±4	1953	96.9			
Golden Delicious × Lobo	66	389	98.2			
Kosztela × Ligol	55	144	98.6			
Ligol × Arlet	60±4	460	97.6	17.8	12.35	0.005
Ligol × Beauty of Repty	55	375	98.7			
Ligol × Braeburn	66	1294	96.1			
Ligol × Fuji	66	245	95.5	23.1	0.46	0.5
Ligol × Gala	54	552	96.9			
Ligol × Gala Must	60±4	259	98.8			
Ligol × Granny Smith	66	1008	96.7			
Ligol × Kosztela	60±4	177	98.9			
Ligol × King of the Pippins	55	192	98.4	25.9	0.09	0.8
Ligol × Lobo	66	1406	97.3			
Ligol × Priam	66	374	97.9	18.3	8.75	0.005
Ligol × Rubin	60±4	857	98.2			
Priscilla × Topaz	60±4	311	99.0			
Redspur × Lobo	55	102	100			
Rubin × Braeburn	60±4	535	97.4			
Rubin × Linda	60±4	243	98.8			
Rubin × Odra	55	113	99.1			
Rubin × Pinova	60±4	185	97.8			
Rubin × U 1165	55	144	95.8			
Rubin × U 641	60±4	443	98.4			
Rubin × Vanda	60±4	427	99.8			
Total		**12650**				
Average			**97.8**	**21.3**		

Table 3. Selected characteristics of apple progenies in 2006

Progeny	Days of additional stratification at 2-3°C	No. of seeds	Seedling emergence [%]	Pale green lethal seedlings [%]	χ^2 3:1	p
Alwa × Topaz	93	78	92.3			
Maigold × Topaz	93	84	98.8			
Total		**162**				
Average			**95.6**			

The seeds of 2003/2004 (year of pollination/year of germination) stratified additionally for 43, 46, 49, 53, 58, 60 and 72 days emerged at 97.3%, 95.6%, 96.5% (97.5% excluding 'Fuji' seeds), 89.5% (97.3% excluding 'Fiesta' seeds), 95.1%, 94.5% (96.9% excluding 'Ligol' seeds) and 96.9%, respectively (Tab. 1). Then, the seeds of 2004/2005 after complementary stratification for 55, 66 and a period of 60 ± 4 (56-64) days produced 97.9% (98.6% excluding 'Fiesta' seeds), 97.0% and 98.3% of seedlings, respectively (Tab. 2). The seeds of 2005/2006 stratified for 93 days emerged at 95.6% (Tab. 3). In some families, a number of seedlings emerged during that time; relatively many were seen in the family 'Ligol' × 'U 641' after 72 days of additional stratification.

Pale green lethal seedlings occurred in 10 families of the following parent cultivars: 'Arlet', 'Delbarestivale', 'Fuji', 'King of the Pippins', 'Ligol', 'Priam', 'Rubinola', 'Redfree' and 'Šampion'. The percentage of lethal seedlings ranged from 17.6% to 31.3% in 2004 (Tab. 1), and from 17.8% to 25.9% in 2005 (Tab. 2). In 7 crosses, segregation of normal vs. lethal seedlings agreed well with a 3 : 1 ratio. Three crosses yielded pale green lethal seedlings in amounts that did not meet expectations, but the results were probably within the limits of the sampling error (Klein et al., 1961).

The mortality of seedlings due to the pale green lethal gene *l* was absent in the populations of the cultivars 'Arlet', 'Delbarestivale', 'Fuji', 'Golden Delicious', 'King of the Pippins', 'Ligol', 'Priam', 'Prima', 'Rubinola', 'Redfree' and 'Šampion' crossed with 'Beauty of Repty', 'Bolero', 'Braeburn', 'Cox's Orange Pippin', 'Fiesta', 'Florina', 'Gala', 'Gala Must', 'Granny Smith', 'Idared', 'Kosztela', 'Lobo', 'McIntosh Wijcik', 'Otava', 'Priscilla', 'Redspur', 'Rubin', 'Spartan', 'Spartan 2/8', 'Topaz' and 'U 641' (Tab. 1, 2).

DISCUSSION

Seedling emergence obtained in our experiment with the new method of stratification developed by Pitera and Odziemkowski was very high each year (Pitera, 2003; Pitera et al., 2004). The conventional stratification

method used most often in breeding work worldwide (Tancred et al., 1995; Gao and van de Weg, 2006) is somewhat troublesome as germination of seeds is not uniform, so they must be checked frequently and sown successively. The method used by us is free from such limitations and seeds can be sown concurrently (Pitera, 2003).

The influence of parent cultivars on seed germination has been documented by some authors (Tancred et al., 1995, Keulemans et al., 1994; Pitera, 2003; Pitera et al., 2004). In our study, a few cultivars showed differences in seedling emergence. The seeds of 'Fiesta' usually emerged at the lowest rate, irrespective of the pollen parent and season. Three families with 'Ligol' and 'Fuji' as seed parents also produced a relatively low number of seedlings. For both cultivars, however, the rate was higher than 85%, and most of the 'Ligol' populations emerged at an average rate of over 96%. The highest emergence rate was exhibited by the seeds of 'Bancroft', 'Delbard Jubilé', 'Delbarestivale', 'Elstar', 'Golden Delicious', 'Kosztela', 'Maigold', 'Priscilla', 'Redspur', 'Rubin', 'Spartan', and 'Spartan 2/8' (>98%). Previously, very high emergence rates were recorded by Pitera (2003) for 'Golden Delicious', 'Ligol' and 'Šampion'.

The authors of the original method compared 30, 40 and 68 days of complementary stratification (Pitera, 2003; Pitera et al., 2004). They obtained almost the same seedling emergence rate after 40 and 68 days, but not after 30 days as there appeared to be not enough time for some families to germinate at a higher rate (Pitera, 2003). The period applied by us varied between 43 and 92 days, and the differences in seedling emergence were also small.

Generally, our results have confirmed the high effectiveness and simplicity of the new method of stratification, so it should be recommended as an almost ideal method for use in apple breeding.

Only a few reports have been published on the pale green lethal gene in apple. Genotypes of many older cultivars and selections have been identified. Among the cultivars used in our experiment, heterozygotes *Ll* included 'Golden Delicious', 'King of the Pippins', 'Priam' and 'Prima', whereas homozygotes dominant *LL* were 'Florina', 'Gala', 'Cox's Orange Pippin', 'Idared', 'Spartan' and 'U 641' (Klein et al., 1961; Alston, 1976; Way et al., 1976; Pitera, 2003). Our investigation indicated heterozygosity of the following cultivars: 'Arlet', 'Delbarestivale', 'Fuji', 'Ligol', 'Rubinola', 'Redfree' and 'Šampion'. Moreover, such cultivars as 'Beauty of Repty', 'Bolero', 'Braeburn', 'Fiesta', 'Gala Must', 'Granny Smith', 'Kosztela', 'Lobo', 'McIntosh Wijcik', 'Otava', 'Priscilla', 'Redspur', 'Rubin', 'Spartan 2/8' and 'Topaz' are apparently homozygous dominant *LL*. To the authors' knowledge, there has been no report yet on the pale green lethal status of these cultivars. However, homozygosity of cv. 'Fiesta' might be concluded on the basis of its pedigree,

because both parents, 'Cox's Orange Pippin' and 'Idared', are homozygotes (Alston et al., 1987). Also the mutants, 'Gala Must', 'McIntosh Wijcik', 'Redspur' and 'Spartan 2/8', behave identically as their homozygous mother cultivars, 'Gala', 'McIntosh', 'Delicious' and 'Spartan', respectively.

Important genetic implications result from using in crosses cultivars carrying the pale green lethal gene. Alston (1976) has found that gene *l* is closely linked to the *Vf* gene for scab resistance in apple. A consequence of this is a lower percentage of resistant seedlings in progenies of heterozygous cultivars. Other linkages will probably be discovered soon as further attempts have been made (Gao and wan de Weg, 2006).

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WSCHODY I ZAMIERANIE SIEWEK W NIEKTÓRYCH POPULACJACH JABŁONI DOMOWEJ (*Malus* × *domestica* Borkh.)

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S T R E S Z C Z E N I E

Badania prowadzono na 58 rodzinach mieszańców jabłoni w latach 2004-2006. Nasiona do kiełkowania przygotowano stratyfikując je w całych owocach, a następnie uzupełniająco w torfie w wielodoniczkach przez 43-92 dni. Wschody siewek w poszczególnych latach wyniosły średnio powyżej 95%. Wpływ długości okresu stratyfikacji uzupełniającej lub odmian rodzicielskich na procent wschodów był zwykle niewielki. W 10 populacjach wystąpiło zamieranie siewek spowodowane działaniem genu letalnego *l*. Odmiany 'Arlet', 'Delbarestivale', 'Fuji', 'Ligol', 'Redfree', 'Rubinola' i 'Šampion' okazały się heterozygotami *Ll*, natomiast 'Bolero', 'Braeburn', 'Gala Must', 'Granny Smith', 'Kosztela', 'Lobo', 'McIntosh Wijcik', 'Otava', 'Piękna z Rept', 'Priscilla', 'Redspur', 'Rubin', 'Spartan 2/8' i 'Topaz' wydają się być homozygotami *LL*.

Słowa kluczowe: jabłoń, stratyfikacja, wschody, gen letalny *l*