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Evaluation of the Effects of Cluster Numbers Removal on Embryo Emergence of Central Asian Seedless Grape Varieties

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Key words: *Vitis vinifera* L., Stenospermocarpic, Cluster number, Medium, Embryo

Abstract

The potential for embryo rescue is one of the problems in the seedless grape breeding program. The majority of seedless grape types in nature are stenospermocarpic forms, which mostly cause seed abortion after fertilization. In this investigation, four seedless grape cultivars (*Vitis vinifera* L.) were hybridized to evaluate the effects of genotype and removing cluster number on embryo survival. The present findings indicated that removing of clusters does not affect to embryo number whereas the berry number and berry mass were significantly increased, however, a posteriori analysis showed the significance of differences in the number of clusters and berries between Kishnish Sogdiana and Kishmish Terakli varieties only and in the mass of berries - between the raisins of Kishnish Sogdiana and Belya Rosa. Finally, it can be concluded that embryo survival depends on genotype of seedless verities and therefore, the application of embryo rescue should be based on more scientific studies using several varieties of cultivars of grape.

Introduction

The common grape (*Vitis vinifera* L.) has been cultivated for many thousands of years across the globe, including in Uzbekistan, located in the middle of Central Asia. According to N.I. Vavilov, the original location of *Vitis* are the Caucasus and Central Asia regions and also regions as Iran, East China, Asia Minor (Negrul 1946). The many famous and widely cultivated grape cultivars belong to folk breeding in Uzbekistan. Since the late 19th century when grape breeding was begun at the Turkistan Agricultural Experiment Station, a major goal has been to combine certain grape berry attributes such as seed lessness, crisp texture and adherent skin of *Vitis* (Javakyants et. al. 2001). On the

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other hand, a major the grape breeding program in Uzbekistan is orientated on the conventional breeding method and because of recessive genotype of seedless grape male form (♂ seedless X seeded ♀) is used only as a pollinator and result of that is limited to take new seedless varieties and forms (Abdullaev et al. 2020).

The vast majority of seedless grape types are stenospermocarpic, meaning that during fertilization and berry formation the endosperm and seed coat both stop developing properly early on, leaving behind undeveloped seeds or seed traces (Ledbetter et al. 1989). Breeding programs often cross two seedless parental genotypes to increase the likelihood of generating new seedless varieties. In such situations, the progeny generally is obtained through the techniques of embryo rescue with the use of in vitro tissue culture methods (Cain et al. 1983, Jun et al. 2014, Bharathy et al. 2003).

The first application of in ovule embryo culture reported by Emershad and Ramming (1984) where embryo rescue technique has been extensively utilized in grape breeding. There are some reports that rescuing weak and immature plant embryos depends on their stage of maturity and growth medium (Jun et al. 2014). They focused that factor which is constructed on the basis of biology background such as age of the ovule upon removal, cultivars, cross combinations, several genotypes, male parent, pollen parent and some of them studied influence of pre bloom spraying of hormones (benzyl adenine) on *in vitro* recovery of hybrid embryos from crosses (Abdullaev et al. 2020, Jun et al. 2014, Bharathy et al. 2003). It has been demonstrated that stenospermocarpy occurs in several seedless varieties and is stable and unaffected by environmental factors. A demonstrated way to produce seed lessness is the exogenous application of GAs before bloom or during anthesis. It is believed that GAs promotes seedless grapes by inhibiting pollen germination, allowing unfertilized ovules to enlarge and form fruits, as occurs in parthenocarpy (Alejandra et al. 2017). However, another study suggests that exogenous GAs interfere with seed development, as described in stenospermocarpy (Alejandra S et al. 2017, Kimura et al. 1996). So, the mechanism involved in this response is not clear (Alejandra et al. 2017, Cheng et al. 2013).

Surviving of berry seeds while berry ripening stage depends on strongly nutritional balance and agronomical applications play a key role to neutralization of that across the grape bush (Mejía et al. 2011). However, Pedro José Almanza-Merchán et al. (2011), reported as an alternative to improve the quality and production of Riesling x Silvaner berries, retaining 66% of the clusters per plant is recommended, because it generates the highest fruit production per area, mass of clusters and fruits and total solid soluble content, as well as considerable values of total titratable acids and technical maturity index with a low pH value (Candolfi-Vasconcelos and Koblet 1990). Supporting this data, Marko et al. (2014), reported the positive effects of crop removal, including advanced fruit maturity, decreased acidity and increased anthocyanins and phenolics in Cabernet Sauvignon. General increases in berry mass and wine phenols and anthocyanins from Merlot and Cabernet Sauvignon cluster thinning treatments were confirmed by Prajitna et al. 2007.

There are few reports whether agronomical application could affect embryo development and embryo emergence. Furthermore, not only the pollination types, crossing compatibility and genotype potential but also the sensibility within the varieties with respect to removing cluster number is an essential process for embryo emergence in grapevines. Therefore, the present investigations were performed to determine the effects of removal of cluster number on embryo survival of the popular seedless grape cultivars in Uzbekistan including, Belya Roza, Kishmish Rozoviy, Kishmish Sogdiana and Kishmish Terakli.

Materials and Methods

The breeding process of the present experiment was carried out in 2020 at the Samarqand branch of the Scientific-Research Institute of Horticulture, Viticulture and Winemaking named after Academician M. Mirzaev. Four accessions of seedless grapes *Vitis vinifera* L. (Kishmish Sogdiana, Kishmish Terakli, Kishmis rozoviy and Belya roza) from the grapevine germplasm collection of SRIHV&W-Samarqand (Mirzaev 1984), with specific characteristics were crossed during this study (Table 1).

Table 1. Cross-combination Central Asian seedless cultivars.

Cross combination			
Belya Roza ♀	Kishmish Sogdiana ♀	Kishmish Terakli ♀	Kishmish Rozoviy ♀
Kishmish Sogdiana ♂	Belaya Roza ♂	Kishmish Sogdiana ♂	Kishmish Sogdiana ♂
Kishmish Terakli ♂	Kishmish Terakli ♂	Belaya Roza ♂	Kishmish Terakli ♂
Kishmish Rozoviy ♂	Kishmish Rozoviy ♂	Kishmish Rozoviy ♂	Belaya Roza ♂

The vines were all 12 years old and trained to a V-trellis with a planting density of 2.5 × 3m. The cultivars that have been included study were selected on the basis of their blooming time and this period lasted from May 23 to June 1 of 2020 year (Negrul 1952, Larkin et al. 2015, Javakyants et al. 2001).

The surface of grape clusters was sprayed with 70% ethanol solution before the emasculation procedure (Martinelli et al. 2001). The emasculation procedure was carried out in the early time from 5:00 am to 10:00 am, while with temperature touched at 25°C the procedure was finished. After normalizing the water balance of the cluster and water inflows on it from 17:00 to 19:00 we continued the emasculation technique. Second part of emasculation process (17:00 -19:00 pm), inflorescences separated from calyptra was removed from the cluster of grapes. Emasculation was done by removing the cap and stamens, with sterile a fine-point forceps (Javakyants et al. 2001, Negrul 1936). Before anthesis the emasculated cluster of grapes was isolated with double layer white paper bags, light transmission is 58% (WPB) on May 24-26 to prevent contamination by foreign pollens, three days before breeding procedure. The Central Asian seedless grape verities of Kishmish Sogdiana, Kishmish Terakli, Kishmis rozoviy, Belya Roza and seeded grape verity of Toyfi were selected for breeding program. The blooming time of all five verities lasted from May 23 to June 3 in the Samarqand region condition. The cross-breeding procedure was organized form May 29 to June 1. All seedless cultivars Kishmish Sogdiana, Kishmish Terakli, Kishmis rozoviy, Belya Roza were crossed with seeded Toyfi verity, which was selected as the male form. The female forms of four seedless cultivars were pollinated by placing fresh pollen directly into stigmas. Pollinated clusters were isolated with WPB to exclude random pollination and then was allowed to develop. All process was organized in the early morning from 5:00 am to 8:00 am, while temperature upgraded till 20°C. All selected grape verities have bisexual flowers, and blooming time is occurred in the same time of blooming season (Smirnov et al. 1995).

Grape berry disinfection and embryo rescue. The grape clusters were harvested at different times, 30-40-80 days after pollination (DAP), for ovule excision from the grapevine germplasm collection of SRIHV&W-Samarqand. Grape berries were transferred to the laboratory of “Transgenomics and tissue culture” of G&B center (Tashkent). The surface of berries was washed in running tap water to remove mud and dirt (Ksenija JT et al. 2005). The sterilization of berries started by treating the berries with a dip in 96% ethanol for 40 s into laminar box (HFsafe LC), followed by NaClO 4% for 10 min and three times washing in sterilised water. Sterile berries were separated into two parts inside of Petri dishes and were stored at 4°C condition for 24 hrs. Conserved berries rudiments under 4°C condition was transferred on medium (Emershad et al. 1994).

For all statistical analysis, R software version 4.0.2 was used. The effects of factors (Flower clusters and Varieties) on the variability of quantitative variables had been evaluated using analysis of variance (ANOVA). The Scheffe's test was applied when there were significant differences ($\alpha = 0.05$).

Results and Discussion

In the 2020 experimental year, the blooming time was observed on 3rd decade of May. The observed cultivars demonstrated diverse resistance reactions against unsuitable climate condition. For instance, Belya Roza cultivar reformed up to 8 inflorescence or flower clusters per bush, in the other two replications flower clusters were 7 per bush. Kishmish Sogdiana seedless cultivar had the same numbers of fluorescence in three replications a mere 8, whereas Kishmis rozoviy cultivars had 6, 7 and 9 of it, in the 1st, 2nd and 3rd replications. For instance, Kishmish Sogdiana demonstrated high sensibility of cluster formation within the group, with the mean berries number being control 240 (SD ± 4.89), in the second variant 242 (SD ± 4.30) and last application 11 fewer grape berries that control 229 (SD6.90) (Fig. 1).

Kishmish Rozoviy and Belaya Roza cultivars demonstrated lower sensibility of reducing flower cluster per bush. It can be seen (Fig. 1) that berry number decreased up to 8 berries (163.3333 ± 8.04) by first cultivar and five by second 181.6667 ± 6.46 compared with control 171.8696 ± 2.20 and last 186.0909 ± 3.94. Some scientists, as Mota et al. (2010) (Merchán et al. 2011) found that the thinning of clusters generated a decrease of the cluster weight of Merlot and Cabernet Sauvignon vine grapes. In contrast, Reynolds et al. (1994) reported an increase in the cluster weight, whereas Nuzzo and Matthews (2006) did not find differences. It is observed in our study that cluster formation impacts berry mass and berry number significantly (Table 2).

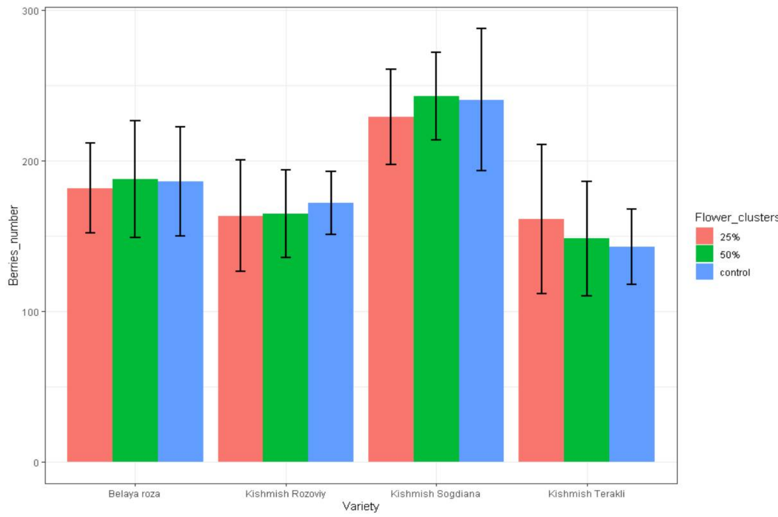

Fig. 1. Berries number for 4 seedless cultivars of *Vitis vinifera* L. across seasons. Histograms of cluster number mean of each genotype are shown. The *blue boxes* indicate the control where bush without removing clusters; the *green boxes* indicate clusters 50% removing of cluster from bush, the *red boxes 25% of cluster thinning*.

Table 2. Berry number four seedless cv per cluster.

Percent of cluster formation per grape bush	Belaya Roza	Kishmish Rozoviy	Kishmish Sogdiana	Kishmish Terakli
	Berry number per cluster of grapes			
Control 100%	186.0909 ± 3.94	171.8696 ± 2.20	240.3750 ± 4.89	142.8125 ± 3.19
50%	187.5455 ± 6.03	164.5455 ± 4.53	242.7500 ± 4.30	148.1250 ± 6.99
25%	181.6667 ± 6.46	163.3333 ± 8.04	229.0000 ± 6.90	161.2500 ± 13.70

Clusters per bush were reduced by cluster remove treatments by 25% and 50% for all four cultivars. The recovery of embryos was selected 30-40-80 days after pollination (DAP). It is obvious that decreasing of cluster numbers per bush increased the grape berry mass in season by all cultivars. We can see from Table 3 that cluster removing generated extremely significant differences (Scheffe's test was used α = 0.05) in berry mass rate (Table 3).

Table 3. Berry weight increasing in three DAP periods (day after pollination 30-40-80) seedless cv.

1. Variety = Belaya roza, gr			
Flower_clusters	30 DAP	40 DAP	80 DAP
Removed cluster 25%	0.6516667 ± 0.012	1.2666667 ± 0.019	6.0966667 ± 0.156
Removed cluster 50%	0.5463636 ± 0.013	1.0109091 ± 0.026	3.6045455 ± 0.104
Control	0.3399091 ± 0.015	0.8963636 ± 0.019	2.9200455 ± 0.112
2. Variety = Kishmish Rozoviy, gr			
Removed cluster 25%	0.5600000 ± 0.012	0.9216667 ± 0.026	2.6116667 ± 0.025
Removed cluster 50%	0.4327273 ± 0.007	0.6363636 ± 0.019	2.4390909 ± 0.064
Control	0.2913043 ± 0.005	0.4754783 ± 0.010	2.0547826 ± 0.036
3. Variety = Kishmish Sogdiana, gr			
Removed cluster 25%	1.7500000 ± 0.042	2.5233333 ± 0.032	6.4316667 ± 0.042
Removed cluster 50%	1.5166667 ± 0.032	2.2733333 ± 0.045	6.0650000 ± 0.152
Control	1.2083333 ± 0.028	1.8962500 ± 0.032	5.2000000 ± 0.173
4. Variety = Kishmish Terakli, gr			
Removed cluster 25%	0.7000000 ± 0.091	1.1275000 ± 0.045	5.2325000 ± 0.041
Removed cluster 50%	0.4625000 ± 0.026	1.0000000 ± 0.029	4.9362500 ± 0.060
control	0.3312500 ± 0.026	0.8156250 ± 0.029	4.3518750 ± 0.078

Berry sizes have increased by all verities. When grape clusters have been reduced to 50%, the Belya Roza cultivar increased to 0.5463636 gr, 0.21 gr heavier than control 0.3399091 ± 0.015 in 30 DAP. Similarly, the berry mass of grape increased with reducing cluster number up to 25%, 32 gr more mass compared with control 0.6516667 ± 0.012. The investigated four grape cultivars always gave the lowest berry size in all control variants. Kishmish Rozoviy in the 30 DAP in the control variants weighted 0.2913043 ± 0.005 gr, after ten days or in the day of 40 DAP enlarged approximately 0.4754783 ± 0.010 the maximum size recorded nearly to harvest time or 80 DAP 2.0547826 ± 0.036 gr, whereas 2nd variant demonstrated other sight, berry size increased significantly in 30 DAP and reached 0.5600000 gr ± 0.012, in 40 DAP-0.9216667 gr ± 0.026 and last period in 80 DAP-2.6116667 gr ± 0.025555. Although a statistically diverse result was obtained with CN 25-50% of the clusters as compared to retaining 100% of clusters, this high fruit number can negatively affect plant growth and diminish the nutritional reserves for future crops.

Kishmish Sogdiana demonstrated high sensibility among three other cultivars to cluster thinning. In the control variant berry mass measured approximately up to 1.2 gr in 30 DAP period and taking berry mass was continued till the end of season from, 1.89 gr in 40 DAP until 5.2 in 80 DAP. Significantly increasing was observed in 25% cluster thinning in 30 DAP berry mass was 0.55 gr heavier than control variant (1.2083333 ± 0.028 to 1.7500000 ± 0.042) and in the last period 6.4316667 ± 0.042 compared with control. All thinning treatments increased the berry weight especially in CN 25% treatment providing the highest berry weight in all four seedless.

In the first phase of berry growth, with the Kishmish Terakli variety, cluster thining affected the berry growth, causing rushed in development compared to those of the control. However, before veraison, 25% thinning of clusters showed a higher average berry weight with significant differences (Fig. 2).

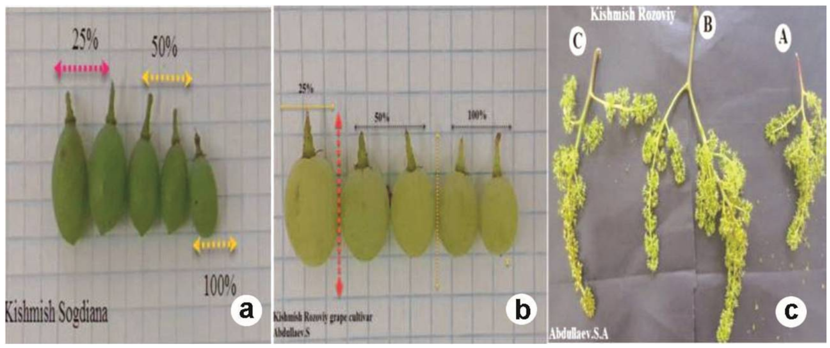

Fig. 2. Inflorescence of Kishmish Rozoviy cultivars (a) Kishmish Sogdiana, (b) Kishmish Rozoviy cvs berry size in the period of 40 DAP, by control (form right to left) - 25% - 50% CN and (c) 1st June bloom period, A - 25% cluster thinning, B - control, C - 50% cluster thinning.

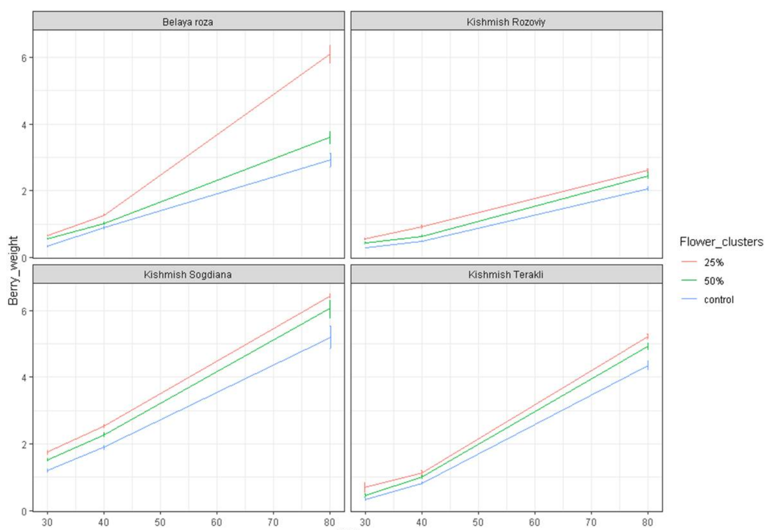

Fig 3. Effects of cluster thinning, with 25%, 50% or 100% retention of clusters per plant, in four seedless varieties grapevine on: berry mass per cluster: To study the significant differences, the Scheffe's test was used (α = 0.05).

When compared to the control, the cluster thinning treatment (50) also altered the berry's growth during the ripening stage. Therefore, only the Kishmish Sogdiana exhibited a maximum berry mass during harvest, according to the Belaya Roza cultivar, which significantly differed among other cultivars (Fig. 3).

We selected 30-40-80 DAP for estimating effects of cluster numbers formation on embryo emergence percent. The basal medium for experiments were prepared Erikson and Anderson with activated charcoal growth medium. In the experiment period the berry rudiments rate of embryo for seedless cultivars of all Kishmish Sogdiana, Kishmish Terakli, Kishmis rozoviy and Belya roza were dependent on genotype (Table 4).

Table 4. Embryo emergence for four seedless cultivars.

Cultivars	Berries number	Rudiment number	Number of rescued embryo	Percent %
Belaya Roza	Control			
	4094	4994	578	11.5739
	1st variant - 50%			
	1810	2710	253	9.33579
	2nd variant - 25%			
	980	1880	146	7.76596
Kishmish Rozoviy	Control			
	3953	4853	435	8.96353
	1st variant - 50%			
	2913	3813	279	7.31707
	2nd variant - 25%			
	1374	2274	153	6.72823
Kishmish Sogdiana	Control			
	5568	6468	732	11.3173
	1st variant - 50%			
	1185	2085	223	10.6954
	2nd variant - 25%			
	645	1545	132	8.54369
Kishmish Terakli	Control			
	2285	3185	96	3.01413
	1st variant - 50%			
	1185	2085	53	2.54197
	2nd variant - 25%			
	645	1545	23	1.48867

Negrul (1946) divided seedless cultivars into 4 categories depending on rudiment mass, 1st category-0-6 mg, 2nd category - 6.1-10 mg, 3rd category - 10.1-14 mg, 4th category - 14.1 mg. In the experiment period, the grape berry quality was not evaluated; the rudiment number per berry ranged from 1 to 3.5 in the control and the other two variants (Fig. 4).

Cluster thinning treatment significantly affected embryo numbers in all cultivars. Belya Roza, which took maximal berry weight up to 6.0966667 gr ± 0.156 in 25% cluster had lower embryo percent a mere 7.7%. This treatment doubled the rudiment number compared to control from 980 grape berries 1880 rudiments, on the other hand, decreased embryo number rapidly to 3.8% and compared with control 11.5-7.7%. Kishmish Rozoviy cultivar increased up to 1.66% (2774 rudiments from 1374 berries) rudiment number in 25% cluster number compared with control (4853r = 3953b) (Fig. 4). The present findings indicated that the cluster thinning caused an increased rudiment number and reduced embryo numbers ($R^2 = 0.0068$). Cluster numbers 50% and 25% have reversed embryo emergence level to values within the control group. Kishmish Terakli cultivars had a lower percent of embryo numbers in groups from 2285 grape berries had 3185 rudiments and of all them were rescued 96 embryos or embryo formation rate was 3.01%. Rudiment numbers in 50% cluster variant increased 0.36% percent on the other hand application decreased embryo number up to 2.5 % or the number of embryos was 43 fewer compared to control 96-53.

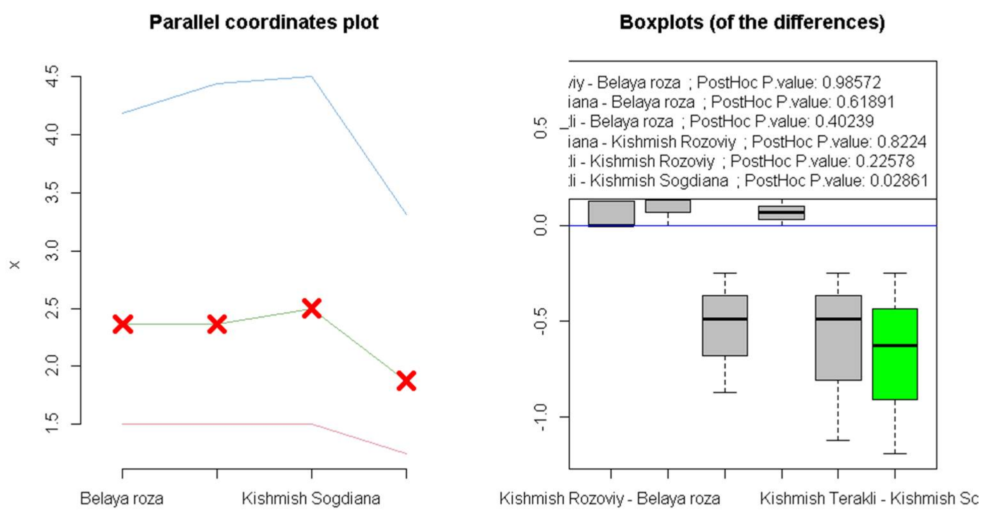

Fig 4. Friedman test results matched the two-way ANOVA. The difference between cultivars at different thinning rates was significant for the mean of all traits. However, a posteriori analysis showed the significance of differences in the number of clusters and berries between only Kishnish Sogdiana and Kishmish Terakli varieties and in the weight of berries - between the raisins of Kishnish Sogdiana and Belya Rosa.

The difference in size between seeded and seedless a berry is a consequence of their cellular development. Larger berries have larger mesocarp cells, regardless of berry classification. The difference in size between seeded and seedless berries is a consequence of their cellular development. Larger berries have larger mesocarp cells, regardless of berry classification (Fig. 6). At 40 DAP the endosperm cells were relatively less and the number of abnormal embryos were significantly more (Fig. 7). Kishmish Sogdiana had larger mesocarp cells and lower percent of empty embryos sac and followed Belaya Roza grape sort. To investigate the status of embryo development, we found that the small global embryo had developed at 40 DAP. The number of embryos slightly decreased in 25% and 50% cluster number. Especially, lower percent of embryos formation was observed by 25% cluster thinning, among cultivars by Kishmish Terakli.

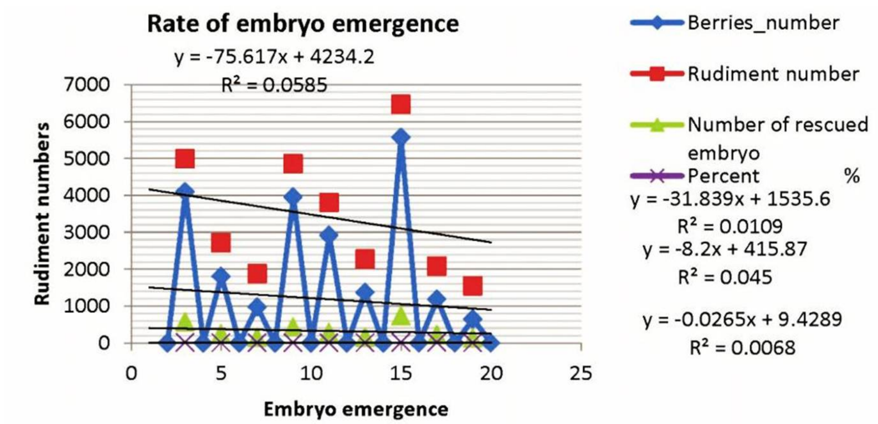

Fig 5. Rate of rudiment and embryo numbers. In application increased berry and rudiment numbers but decreased embryo numbers in all seedless groups. Longitude of grape embryo in 40 DAP (Kishmish Terakli), A - existing embryo, B - embryo sac without embryo.

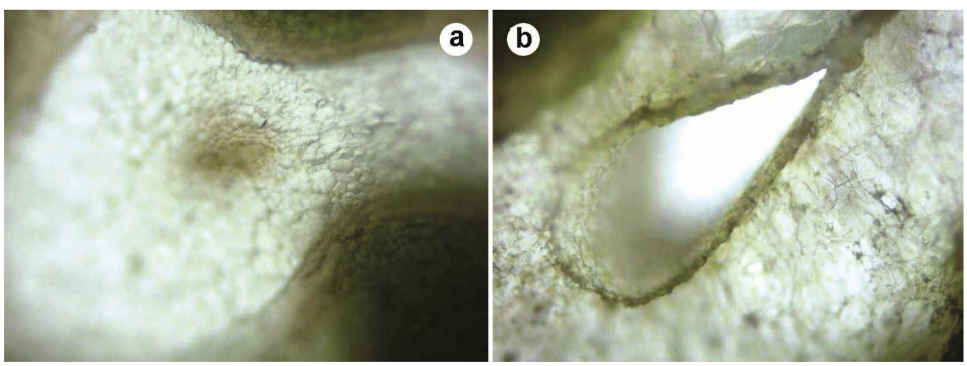

Fig 6. Two different embryos obtained from seedless cv in 40 DAP (Kishmish Terakli): (a) Existing embryo and (b) Embryo sac without embryo.

The data observed in this study support the previous reports that positive effects of crop removal, including advanced fruit maturity, decreased acidity and increased anthocyanins and phenolics in Cabernet Sauvignon (Mejía et al. 2011, Pedro et al. 2011, Candolfi-Vasconcelos et al. 1990). Our results are in line with the data of Marko et al. (2014) who reported clusters thinning treatment by ~ 40% for cultivar Merlot and by ~ 50% for cv. Cabernet Sauvignon increased Merlot cluster weight by ~ 14%, while Cabernet Sauvignon cluster weight was increased by more than 25%. All thinning treatments increased the berry weight, especially clusters thinning + berry clusters thinning treatment providing the highest berry weight in both years and both cultivars. Similarly, to our data CT treatment by ~ 25% for cultivar Belaya Roza and by ~ 50% for cv. Kishmish Sogdiana berry weight increased significantly. There are some interesting facts that Mota et al. (2010), found that the thinning of clusters generated a decrease of the cluster weight of Merlot and Cabernet Sauvignon vine grapes. On the other hand,

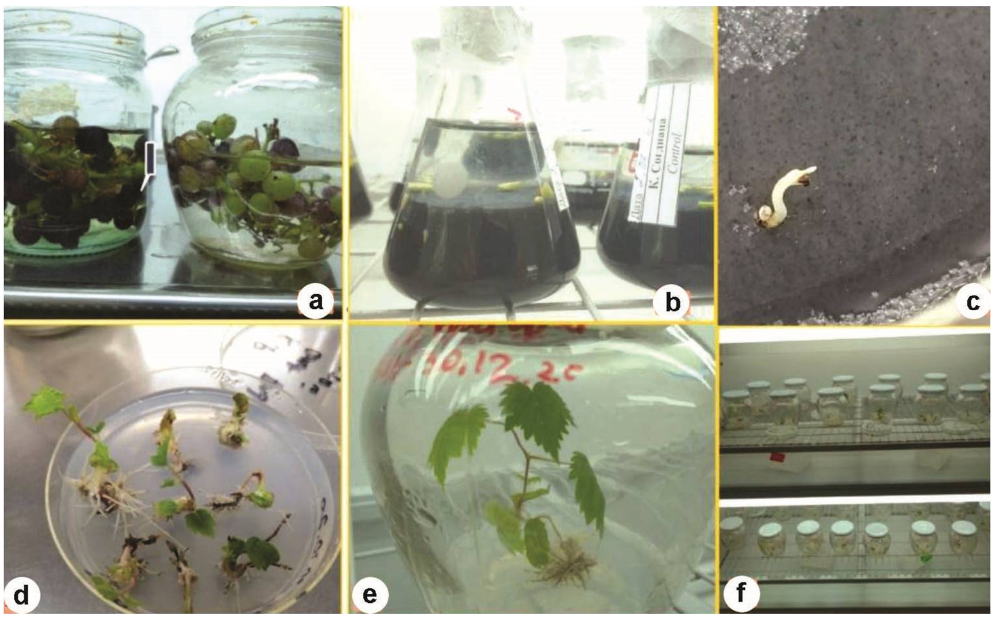

Fig. 7. Embryo rescue method of seedless cultivars: (a) Surface sterilization, (b) Erikson media for rudiments, (c) Embryo development, (d) Planlet of grapes in shooting period, (e) Rooting period of hybrid generation and (f) Grow room in special medium.

Reynolds et al. (1994) reported an increase in the cluster weight, whereas Nuzzo and Matthews (2006) did not find differences (Marko et al. 2014, Merchán et al. 2011, Mota et al. 2010, Reynolds et al. 1994, Nuzzo et al. 2006). In our study, increasing of berry size by all for verities was observed. When grape clusters have been reduced by ~ 50% by Belya Roza berry weight cultivar increased to 0.5463636 gr 0.21 gr heavier than control 0.3399091 ± 0.015 in 30 DAP. Similarly, the berry weight of grape increased with reducing cluster number by ~ 25, 32 gr more mass compared with control 0.6516667 ± 0.012. Kishmish Sogdiana demonstrated high sensibility among three other cultivars to cluster thinning. In the control variant berry mass measured approximately up to1.2 gr in 30 DAP period by control and taking berry mass was continued till end of season from 1.89 gr in 40 DAP until 5.2 in 80 DAP when mass obtained by ~ 50% 1.5166667 gr ± 0.032, 2.2733333 gr ± 0.045 and 6.0650000 gr ± 0.152.

For embryo rescue culture depends also on, identifying optimal time of mature embryos is important. Gray et al. (1992) cultured ovules from pollinated berries of the Orlando Seedless variety sampled at 10, 20, 40 and 60 days after pollination and found that more embryos and plants were recovered from cultured ovules sampled at 40 or 60 days. It has also been reported that more embryos can be obtained from ovules cultured at 60 and 70 days after flowering (DAF), although the most vigorous growth in this study was observed from ovules cultured at 30 and 43 DAF (Jun et al. 2014, Gray 1992).

Our preliminary study showed that the best time to pursue an embryo rescue was 80 DAP of four cultivars. At 30 and 40 DAP, abnormal ovules were observed in the berry. At 80 DAP, full browning and estimated normal embryos were observed. To further test in detail, we conducted a systematic investigation on the embryo abortion on this seedless variety. This optimal time for embryo rescue was determined by sampling ovules at 80 DAP. Interestingly is that all four Central Asian cultivars demonstrated different embryo numbers. Cluster removing had negatively effect to embryo survival. In the group Belya Roza cv. Demonstrated high rate of normal embryos 11.57% at 80 DAP and treatment of CT decreased embryo quantity up to 9.3 % by ~ 50% and 7.7% by ~ 25% CT. Second cv. among the group was Kishmish Sogdiana, at 80 DAP from 6468 rudiments were rescued 732 embryos that is equal to 11.3173% and by ~ 50% CT 10.6954% and by ~ 25% only 8.54369%. Kishmish Terakli seedless cv demonstrated the lowest percent of embryo emergence in both 50% ~ 25% CT and control. From 3185 rudiments were 96 embryos transferred to medium or only 3.01% in control and by ~ 50% embryos equaled to 2.5% or from 2085 rudiments 53 embryos, the lowest rate showed by ~ 25% only 1.4% or from 1545 rudiments only 23 embryos.

A demonstrated way to produce seedlessness is the exogenous application of gas before bloom or during anthesis (Alejandra et al. 2017). It is believed that Gas promotes seedless grapes by inhibiting pollen germination, allowing unfertilised ovules to enlarge and form fruits, as occurs in parthenocarpy (Alejandra et al. 2017, Kimura et al. 1996). However, another study suggests that exogenous GAs interfere with seed development, as described in stenospermocarpy (Alejandra et al. 2017, Cheng et al. 2013). So, the mechanism involved in this response is not clear (Alejandra et al. 2017).

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