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Determination Of The Suitable Nutrient Medium For the Immature Embryo Culture In Different Genotypes of Safflower (*Carthamus tinctorius* L.)

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ABSTRACT

Despite its adequacy in many agricultural products, Türkiye has a large deficit in vegetable oil production. For this reason, it is necessary to focus on the studies conducted on new oil crops that will contribute to vegetable oil production and be effective in eliminating our oil deficit. Safflower is one of the plants that make up this group. In this study, it was aimed to determine the most suitable nutrient medium in order to speed up the obtaining homozigot lines in the breeding programs of safflower through the immature embryo culture technique in different Safflower genotypes. The study was carried out at the Speed Breeding Center of the Eastern Mediterranean Agricultural Research Institute. In the study, five safflower genotypes (Line-1, Line-2, Line-3, Line-4 and Dinçer) and three media (MS, NN and B5) were tested. Immature embryos isolated 14 days after pollination from the seeds of plants grown under open field conditions were tested. Plant weight, plant root length, plant height and number of leaves per plant were investigated in the regenerated plants from the cultured embryos. The research findings showed that the average values of plant weight, plant root length, plant height, number of leaves per plantlet and regeneration rate varied as 0.15-0.28 g, 2.22-2.84 cm, 1.59-1.80 pieces, 1.35-1.59 cm, respectively, depending on the media. The averaged values of the mentioned characteristics varied as 0.14-0.27 g, 1.41-1.61 cm, 2.09-3.12, 1.53-1.82 pieces and 0.8-33.8% respectively, depending on the genotypes. Based on the research results, it was concluded that the immature embryo culture technique could be used to accelerate the obtainment of homozygous lines in safflower breeding, that the genotype and nutrient medium significantly affect the application efficiency of the technique, and that the nutrient medium to be used is dependent on the genotype.

Farklı Aspir (*Carthamus tinctorius* L.) Genotiplerinde Olgunlaşmamış Embriyo Kültürü İçinUygun Besi Ortamının Belirlenmesi

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ÖZET

Türkiye, birçok tarımsal üründeki yeterliliğine karşın, bitkisel yağ üretiminde büyük açık vermektedir. Yağ bitkileri ve bitkisel yağ üretimimiz tüketimimizi karşılayamadığı için her yıl ham ve rafine yağ ile yağlı tohum küspesi olarak üç milyar doların üzerinde yağlı tohum ithalatı yapılmaktadır. Türkiye'nin bitkisel yağ açığı her geçen yıl ürkütücü boyutlarda artış göstermektedir. Oysa, Türkiye ekolojisi pek çok yağ bitkisi için geniş bir üretim potansiyeline sahiptir. Bu potansiyel harekete geçirilebilirse, Türkiye bitkisel yağ ithalatçısı ülkeler arasından sıyrılarak, kolaylıkla bitkisel yağ ihracatçısı ülkeler arasına girebilir. Bu nedenle bitkisel yağ üretimine katkıda bulunacak ve yağ açığımızın giderilmesinde etkili olacak yeni yağ bitkileri üzerinde önemle durulması gerekir. Aspir bitkisi de bu grubu oluşturan bitkilerden birisidir. Bu araştırmada, farklı aspir (Carthamus tinctorius L.) genotiplerinde olgunlaşmamış embriyo kültürü tekniğiyle homozigot hatların elde edilmesini hızlandırabilmek amacıyla en uygun besi ortamının belirlenmesi amaçlanmıştır. Çalışma Doğu Akdeniz Tarımsal Araştırma Enstitüsü bünyesindeki Generasyon Atlatma Merkezi'nde yürütülmüştür. Çalışmada material olarak dört aspir hattı (Hat-1, Hat-2, Hat-3, Hat-4 ve bir aspir çeşidi (Dinçer) olmak üzere beş aspir genotipi ve üç besi ortamı (MS, NN ve B5) test edilmiştir. Açık arazi koşullarında yetiştirilen bitkilerden tozlanmadan 14 gün sonra alınan olgunlaşmamış embriyolar in vitro koşullarda test edilen besi ortamlarında kültüre alınmıştır. Kültüre alınan embriyolardan rejenere olan bitkilerde bitki ağırlığı, bitki kök uzunluğu, bitki boyu ve bitki başına yaprak sayısı incelenmiştir. Araştırma bulguları, incelenen besi ortamlarında ortalama bitki ağırlığı, bitki kök uzunluğu, bitki boyu bitkicik başına yaprak sayısı ve rejenarasyon oranı değerleri sırasıyle 0.15-0.28 g, 2.22-2.84 cm, 1.59-1.80 adet, 1.35-1.59 cm arasında değiştiğini, genotiplere bağlı olarak ise bu değerlerin, 0.14-0.27 g, 1.41-1.61 cm, 2.09-3.12 ve 1.53-1.82 adet ve % 0.8-33.8 olarak değiştiğini göstermiştir. Araştırma sonuçlarına dayanarak, olgunlaşmamış embriyo kültürü tekniğinin aspir ıslahında homozigot hatların elde edilmesinin hızlandırılmasında kulllanılabileceği, genotip ve besi ortamının tekniğin uygulanma etkinliğini önemli derecede etkilediği, kullanılacak besi ortamının genotipe bağımlılık gösterdiği sonucuna varılmıştır.

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INTRODUCTION

Safflower (*Carthamus tinctorius L.*) is an annual oilseed crop originating from Eurasia, including Türkiye and its neighbouring countries (Esendal 2001). The oil content of safflower seeds ranges of 30-45%, making it valuable for both food and industrial applications. In addition to oil production, safflower seeds are commonly used as bird feed, and its fresh flowers contribute to high- quality honey production. Interspecific hybridization studies have been conducted to broaden the genetic base of this crop species.

Safflower oil is used both as cooking oil and is preferred in biodiesel production. It is also a plant that is used in various sectors such as margarine, paint, varnish, pharmaceutical and feed industry. The dye obtained from safflower flowers is often used by mixing it with saffron. Safflower plant has great importance in cultivation due to its advantages such as being resistant to drought conditions, being resistant to diseases and pests, and being suitable for mechanized agriculture. It also plays a major role in the evaluation of dry farming areas. The pulp remaining after the oil is extracted is used as animal feed due to its 22-24% crude protein content (Babaoğlu, 2007; Demir and Karaca, 2018).

Safflower is a valuable oil plant that can be easily grown in difficult land conditions and contains 30-45% oil in its seeds (Eryılmaz *et al.,* 2014*;* Serim *et al.,* 2015). The total unsaturated fatty acid ratio, which is important for human health, is quite high, usually between 90-93%. There are two different types of safflower oil. The first one contains a high level of linoleic acid (Omega-6) and its fatty acid ratio is 78%. This type is generally used in the chemical and feed industries. The other one contains a high level of oleic acid (Omega-9) and is a high-quality cooking oil. Varieties with an oleic acid ratio of approximately 85% have been developed *(*Babaoğlu, 2006).

Safflower plant has a bright future in closing the oil gap in our country. According to TUİK 2023 data, it has been produced in our country with 39000 tons and it is known that the production in Kırşehir province where the trial was conducted is 3639 tons (TUİK, 2023). According to FAO 2022 data, safflower production in the world is 995507 tons and it is known that the production is mostly in the Asian continent with 599103 tons. Kazakhstan ranks first as the country with the highest production with 447456 tons (FAO, 2022).

In order to broaden the gene pool of safflower, interspecific crossings are possible. However, development of hybrid embryos is impossible due to the differences between parental genomes in some cases (*C.tinctorius* 2n=24 *C. lanatus* 2n=44). Survival rate of F1 plants is low when the chromosome numbers of hybrid plants are different (Golkar 2014). It is needed to develop the embryo rescue technique to overcome genetic and cytological barrier after hybridization between wild and cultivated species. On the other hand, obtaining the homozigot lines of safflower in the breeding program is possible to speed up through the immature embryo culture.

In this research, effects of different media on the regeneration rate of the immature embryos of genotypes were examined.

MATERIALS AND METHODS

In the research, four lines of safflower (H1, H2, H3, H4), which are developed in the safflower breeding program of the East Mediterranean Agricultural Research Institute, Adana, and one safflower cultivar (Dincer), which is registered by GAP Agricultural Research Instıtute, Şanlıurfa, were used as plant material. Three different media, MS (Murashige & Skoog, 1962), NN (Nitsch & Nitsch, 1969) and B5 (Gamborg *et al*. 1968), were tested. The pH of the nutrient media was adjusted to 5.8 using 1N KOH and 1N HCl. The sterilization of the nutrient media was provided in the autoclave at 1.2 atmospheric pressure and 121°C for 15 minutes. The laboratory experiment was arranged according to the completely randomized design in split plots with four replications, by taking genotypes as the main

plot and the nutrient medium as sub-plot. 40 embryos were cultured in 4 petri dishes for each genotype - medium combination.

The single safflower receptacle harvested 14 days after pollination was labeled for which genotype it belongs to, and then it was taken into the laboratory environment. In the embryo culture, the embryos in the fruits of the first 5 rows on the outermost part of the receptacle were used. After the fruits separated from each receptacle are subjected to surface sterilization with 70% alcohol for a very short time in a sterile cabinet in a glass container, alcohol is poured and sterilization solution containing 20% bleach (containing 5% sodium hypochlorite, without perfume) + 3-5 drops of Tween 80 was added into the container.

Fruits were sterilized by rinsing in the solution for 10 minutes (Dağüstü *et al.,* 2012). Then, the fruits were rinsed 4-5 times with sterile distilled water and surface sterilization was completed. For embryo isolation and culture of isolated embryos, the skins of fruits subjected to surface sterilization were cut and embryos were removed; after the embryos were removed and separated from the embryo sac, 10 embryos were cultured in each 60 x 15 mm petri dish with 7 ml embryo development medium. All cultures were kept in a climate cabinet providing photoperiod of 20 hours of light (6000-8000 Lux light intensity)/ 4 hours of darkness and 24 ± 2°C temperature conditions. After 3-4 weeks from the beginning of the culture, the embryos that germinated and formed a plant were counted in each petri dish, and the regeneration rate was determined by proportioning the number of embryos that formed plants to the number of embryos cultured in the petri dish. At this stage, plant weight, plant root length and plant height measurements were also taken in regenerated plants.

Variance analysis was applied to the data obtained from the research, using the JMP 7.0 (Copyright © 2007 SAS Institute Inc.) statistical package program according to completely randomized desing in the split plots. The statistically significant factor means were compared with the LSD test.

RESULTS AND DISCUSSION

According to the variance analysis results, sunflower genotypes, nutrient medium and genotype x nutrient medium interaction were found to be significant in all traits examined (Tables 1, 2, 3, 4 and 5).

Regeneration Rate(%)

Genotype, culture medium and genotype x culture medium interaction did statistically significant affect the regeneration rate of immature embryos in the safflower. The mean regeneration rates of immature embryos of different safflower genotypes cultured in different culture mediums are given in Table 1.

As seen in Table 1, regeneration ratio of the embryos isolated from the genotypes showed statistically significant differences between the genotypes. The embryos of the Line-3 showed statistically significant higher reneration rate than those of the other genotypes. The embryos of the Line-1 gave significantly lower reneretaion rate than those of other genotypes. Embryos of the Lin-4 showed significantly higher regeneration rate than tohose of the Line-2 and Dinçer.

As an average of genotypes, B5 and MS media provided statistically significantly higher regeneration rates than the NN medium. However, the statistical significance of genotype x nutrient medium interaction reveals that the effect of nutrient media on regeneration rate of safflower embryos varied significantly depending on the genotypes. In fact, while immature embryos of the Line-4 and Cultivar Dinçer showed statistically significant higher regeneration rate on NN medium than in other media, embryos of the Line-1 cultured on the B5 mediım showed significantly higher regeneration rate

than those on the other medioa. On the other hand, embryos of the Line-2 cultrured on MS medium gave higher regeneraton rate than those on the other media.

Table 1

Averaged Values (%) of the Regeneration Rate of Immature Embryos of the different Safflower Genotypes Cultured on Different Media

Genotipes	B5 Medium		MS Medium		NN Medium		Average	
Line-1	77.7	b[2]	31.0	g	9.0	h	**39.2**	**d***
Line -2	67.0	cd	79.0	ab	8.3	h	**51.4**	**c**
Line -3	75.0	bc	67.0	cd	59.0	de	**67.0**	**a**
Line-4	50.7	ef	46.0	f	87.7	a	**61.4**	**b**
Dinçer	26.7	g	53.7	ef	65.0	d	**48.4**	**c**
Average	**59.4**	**A[1]**	**55.3**	**A**	**45.8**	**B**	**53.5**	
CV(%)	**10.63**	**LSD(0.05)**	**Gen:5.49****		**M:4.25****		**Gen*M:9.51****	

* Mean values with similar low case letters in the same column are not statistically significant different from each other according to the LSD test at $P \leq 0.01$.

[1)] Mean values with similar upper case letters in the same row are not statistically significant different from each other according to the LSD test at $P \leq 0.01$.

[2)] Mean values of genotype-medium comibinations with similar low case letters not statistically significant different from each other according to the LSD test at $P \leq 0.01$.

Dağüstü *et al.* (2012) reported that the transformation rate of sunflower embryos varied between 0.8 and 33.8%, whereas Çil *et al.* (2021a) reported that this rate varied between 56.39 and 72.81%, and again Çil *et al.* (2021b) reported that this rate varied between 26.7 and 100%. It can be said that the different regeneration rates of immature embryos of different species are different due to the differences in the plant species, genotypes and nutrient media tested in the studies.

Plant Height(cm)

According to results of the variance analyses, significant differences were determined at the $P \leq 0.01$ level among the genotypes, media and genotype x nutrient medium combinations in plant height (Table 2). The plant height varied between 1.41 cm and 1.61 cm depending on the genotypes. Plats regenerated from the embryos of the Line-4 showed statistically significant higher averaged plant height than those of the lines 1 and 3. The plants regenerated on the B5 and MS medium showed statistically significant higher plant height that those regenerated on the NN medium. However, significance of the genotype x medium interaction means that effect of the medium on the plant height did significantly varied depending on the genotypes. Thus, embryos of the line 1 cultured on the B5 medium regenerated the plants with significantly higher plant height in comparison with those cultured on MS or NN medium. Embryos of the line 2 cultured on MS medium and those of line 4 on the NN medium gave the plants with significantly higher plant height than those cultured other mediums. On the other hand, embryos of the line 3 cultured NN medium gave the plants with significantly lower plant height than those cultured other medium. Media tested did not significantly changed the plant height of the regenerated plants of the cultivar Dinçer.

Çil *et al*. (2021a) determined the plant height values changing between 1.30 - 4.47 cm sunflower plants regenerated immature emryos of sunflower culturen on different media. They reported that the most suitable nutrient medium was NN nutrient medium. This results are not coincide with those of our study. This disagreement can be explained through the difference of the plant species in the studies.

Table 2

Averaged Values (cm) of the Plant Height of Plants Regenerated from Immature Embryos of the different Safflower Genotypes Cultured on Different Media

Genotipes	Nutrient Mediums							
	B5 Medium		MS Medium		NN Medium		Average	
Line-1	1.60	b-d[2]	1.33	e	1.30	e	**3.12**	**b***
Line -2	1.63	bc	1.90	a	0.93	f	**2.35**	**ab**
Line -3	1.47	c-e	1.63	bc	1.33	e	**2.57**	**b**
Line-4	1.63	bc	1.40	de	1.80	ab	**2.65**	**a**
Dinçer	1.60	b-d	1.60	b-d	1.40	de	**2.09**	**ab**
Average	**1.59**	**A[1]**	**1.57**	**A**	**1.35**	**B**	**2.56**	
CV(%)	**8.21**	**LSD(0.05)**	**Gen:0.13**		**M:0.09****		**Gen*M:0.21****	

* Mean values with similar low case letters in the same column are not statistically significant different from each other according to the LSD test at P ≤0.01.

[1)] Mean values with similar upper case letters in the same row are not statistically significant different from each other according to the LSD test at P ≤0.01.

[2)] Mean values of genotype-medium comibinations with similar low case letters not statistically significant different from each other according to the LSD test at P ≤0.01.

Plant Weight(g)

In terms of plant weight values, the differences between genotypes, nutrient media and genotype x nutrient media interaction values were statistically significant at ≤ 0.01 level (Table 3). In terms of plant weight, the average values obtained from genotypes varied between 0.14 - 0.27 g. The embryos of the line 1 and cultivar Dinçer gave the plants with significatly higher plant weight than those of other lines. On the other hand, The embryos cultured on the NN medium regenerated plants with higher plant weight than those cultured other media. However, significance of genotype x medium interaction showed that the effect of the medium on the plant weight significantly varied depending on the genotypes. Thus, embryos of the Line-1 and culvar Dinçer cultured on NN medium gave the plants with significantly higher weight than those cultured on the other media. The embryos of the Line-2 cultured on MS medium gave the plants with signidicantly hgher plant weight than those cultured NN medium, while the embryos of the Line-3 anmd Line-4 cultured on B5 showed the plants with significantly higher plant weight than those cultured on NN medium.

Table 3

Averaged Values (g) of the Plant Weight of Plants Regenerated from Immature Embryos of the different Safflower Genotypes Cultured on Different Media

Genotipes	Nutrient Mediums							
	B5 Medium		MS Medium		NN Medium		Average	
Line-1	0.15	cd[2]	0.14	c-e	0.50	a	**0.27**	**a***
Line -2	0.16	bc	0.18	b	0.15	cd	**0.16**	**b**
Line -3	0.16	bc	0.15	cd	0.12	de	**0.15**	**bc**
Line-4	0.16	bc	0.14	c-e	0.12	e	**0.14**	**c**
Dinçer	0.15	bc	0.16	bc	0.50	a	**0.27**	**a**
Average	**0.16**	**B[1]**	**0.15**	**B**	**0.28**	**A**	**0.2**	
CV(%)	**8.9**	**LSD(0.05)**	**Gen:0.017****		**M:0.013****		**G*M:0.030****	

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[1)] Mean values with similar upper case letters in the same row are not statistically significant different from each other according to the LSD test at P ≤0.01.

[2)] Mean values of genotype-medium comibinations with similar low case letters not statistically significant different from each other according to the LSD test at P ≤0.01.

Çil *et al.* (2021a) determined that plant weight varied between 0.12 and 0.50 g in the plants regenerated from the embryos of different sunflower genotypes. It was reported that the most suitable nutrient medium was B5 nutrient medium.

Number Of Leaves Per Plant

In the study, genotypes, media and genotype x media interaction did statistically significant affect the number of leaves per plant. The number of leaves per plant varied between 1.53 – 1.82 number depending on the genotypes. Embryos of the Line-1 gave the plants with significantly higher number of leaves per plant than thos of the other genotypes. The plants regenared from the embryos of the cultivar Dinçer showed significantly lower number of leaves per plant than those regenerated from embryos of the other genotypes. The number of leaves per plant varied between 1.59 – 1.80 depending on the medium, and the highest value of the number of leaves per plant was obtained from the plants regenerated from the embryos on B5 nutrient medium (Table 4).

Table 4

Averaged Values of the Number of Leaves Per Plant of Plants Regenerated from Immature Embryos of the different Safflower Genotypes Cultured on Different Media

.Genotipes	Nutrient Mediums							
	B5 Medium		MS Medium		NN Medium		Average	
Line-1	2.03	a[2]	1.77	b	1.67	b-d	1.82	a*
Line -2	1.73	b	1.43	e	1.83	b	1.67	c
Line -3	1.80	b	1.53	c-e	1.77	b	1.70	b
Line-4	1.70	bc	1.70	bc	1.77	b	1.72	b
Dinçer	1.73	b	1.50	de	1.37	e	1.53	d
Average	**1.80**	**A[1]**	**1.59**	**C**	**1.68**	**B**	**1.69**	
CV(%)	**6.69**		**LSD(0.05):**	**Gen:0.21**	**M:0.087***		**Gen*M:0.19**	

* Mean values with similar low case letters in the same column are not statistically significant different from each other according to the LSD test at $P \leq 0.01$.

[1)] Mean values with similar upper case letters in the same row are not statistically significant different from each other according to the LSD test at $P \leq 0.01$.[2)] Mean values of genotype-medium comibinations with similar low case letters not statistically significant different from each other according to the LSD test at $P \leq 0.01$.

[2)] Mean values of genotype-medium comibinations with similar low case letters not statistically significant different from each other according to the LSD test at $P \leq 0.01$.

However, significance of genotype x medium interaction reveals that effect of the media on the number of leaves per plant significantly varied depending on the genoype. Thus, the plants regenerated from the embryos of the Line-1 and cultivar Dinçer cultured on B5 medium gave significantly higher number of the leaves per plant than those cultured on the other media. On the other hand, the plants regenerated from the embryos of the Line-2 and Line 3 cultured on MS medium showed significantly lower number of leaves per plant than those cultured other media. The number of the leaves per plant in the plants regenerated from the embryos of the Line- 4 were not significantly influenced by medium.

Çil *et al.* (2021a) determined that the number of leaves per plant regenerated from the embryos of different sunflower genotypes varied between 1.26 and 3.38. These values are consistent with the plant height values obtained in our study.

Root Length(cm)

According to the results of the variance analyses, significant differences were determined at the 0.01 level among genotypes, nutrient media and genotype x nutrient medium interactions in root length. It was determined that root legth varied between 1.41 – 1.53 cm depending on genotypes, these values

varied between 2.22 - 2.84 cm depending on nutrient medium (Table 5).

Table 5

Averaged Values (cm) of the Root Length of Plants Regenerated from Immature Embryos of the different Safflower Genotypes Cultured on Different Media

Genotipes	Nutrient Mediums							
	B5 Medium		MS Medium		NN Medium		Average	
Line-1	3.73	a[2]	2.97	b-d	2.67	d-g	**3.12**	**a***
Line -2	2.73	c-f	1.30	ı	3.03	bc	**2.35**	**c**
Line -3	2.70	d-g	1.93	h	3.07	b	**2.57**	**b**
Line-4	2.63	e-g	2.50	fg	2.83	b-e	**2.65**	**b**
Dinçer	2.40	g	2.40	g	1.47	ı	**2.09**	**d**
Average	**2.84**	**A[1]**	**2.22**	**C**	**2.61**	**B**	**2.56**	
CV(%)	**6.98**	**LSD(0.05)**	**Gen:0.16****		**M:0.14****		**Gen*M:0.30****	

* Mean values with similar low case letters in the same column are not statistically significant different from each other according to the LSD test at $P \leq 0.01$.

[1)] Mean values with similar upper case letters in the same row are not statistically significant different from each other according to the LSD test at $P \leq 0.01$.

[2)] Mean values of genotype-medium comibinations with similar low case letters not statistically significant different from each other according to the LSD test at $P \leq 0.01$.

Embryos of the Line-1 gave the plants with significantly higher root length thant those of the other genotypes, whie the embryos of the cultivar Dinçer gave the plants with significantly lower root length than those of the other genotype. The embryos cultured on the B5 medium gave the plants with significantly higher root length than those cultured on the otrher media. The plants regenerated from embryos cultured on the MS medium showed significantly lower root length than those regenerated from the embryos cultured on the other media. However, significance of genotype c medium interaction shows that effect of the media on the root length was dependend on the genotypes. Thus, the plants from rhe embryos of the Line-1 cultured on B5 medium showed significantly higher root length than thos regenerated from the embryos on the other media. The plants regnerated from the embryos of Line-2 and Line-3 cultured on MS medium showed significantly lower root length than thos regenerated from the embryos cultured on the other media. The plants regenerated from the embryos of the cultivar Dinçer cultured on NN medium showed significantly lower root length than those regenerated from the embryos cultured on the other media.

Çil *et al.* (2021a) determined that the plant root length varied between 3.52-4.20 in the plants regenerated from the mbryos of the different sunflower genotypes. These values are consistent with the plant height values obtained in our study. Again, Çil *et al.* (2021b) determined that these values varied between 2.66 and 9.25 cm in a similar study they conducted on sunflower.

CONCLUSION

The results of this research showed that the immature embryo culture technique can provide sufflower plants a month after fertilization as compaired to 2-2.5 months after fertilization under in vivo conditions. Thus shortening the reproduction cycle of safflower and thus allowing to obtain 3-5 generations per year instead of 1-2 generations. The results of the research showed that the genotypes of the embryos and nutrient medium are a very important factor in the success of embryo culture technique, but the optimum medium varied depending on the genotype due to significant nutrient medium X genotype interaction.

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