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SELECTION OF PROANTHOCYANIDIN-FREE MUTANTS IN AN IRRADIATED "KAYA" BARLEY POPULATION

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SUMMARY

The clear appearence of a beer is a desired quality factor. Colloidal stability and long-lived brillance of beer is normally achieved by using some additives. Another solution to the haziness problem is to use barley varieties in which the biosynthesis of the proanthocyanidins is generally blocked as a result of the induced mutations. An attempt was made in present study to obtain these kinds of mutants from "Kaya" barley irradiated with gamma rays. M_2- bulk populations were grown at Tokat and Bornova during the 1985-1986. The plants without anthocyanin in the vegetative parts were selected. The individual plants were tested in the progeny rows, two-replicated, at Tokat during the 1986-1987 as M_3. A total of 36 mutant progenies were classified as anthocyanin-less among the progeny rows. Then the seed samples of the anthocyanin-less mutants were analysed for proanthocyanidin content by employing the Vanillin-HCl technique in 1989.

The frequency of visually selected anthocyanin-free mutants was 1.9×10^{-3}. The number of proanthocyanidin-free mutants was 7 based on the chemical analyse thus giving a frequency of 3.7×10^{-4}. It was found that 19 % of the visually selected anthocyanidin-free mutants was also proanthocyanidin-free. The pleiotropic relationship between anthocyanidin and proanthocyanidin producing loci was a genetical basis such an indirect selection.

The biological yield, grain yield, spike number, number of kernels were lower in the proanthocyanidin-free mutants but they were higher than the control for flag leaf area, protein content, early heading, thousand kernel weight and harvest index. The low grain yield and general agronomic preformance of the mutants was in agreement with the previous results. However the higher thousand kernel weight obtained in this study which contradicts the earlier reports could be due to the longer grain filling period as a result of early heading.

There is no great chance of direct usage of these kinds of mutants in production but they could be used in crossing programs.

INTRODUCTION

The clear appearence of a beer is a desired quality factor (1). Permanent and chill haze in beer is due to the precipitation of proteins by polyphenols derived from barley and hop. The barley

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proanthocyanidins constitute the major source of the haze polyphenols. Colloidal stability and long-lived brillance of beer is normally achieved by using enzymes, additives or adsorbents to reduce or remove one of the reactants either the proteins or the proanthocyanidins. Thus the avoidance of precipitation can be accomplished (2-4).

Another solution to the haziness problem is to use barley varieties in which the biosynthesis of the proanthocyanidins is generally blocked as a result of the induced mutations (2-6). Larsen (2) reported that proanthocyanidin-free mutations can be induced in normal varieties after a mutagen treatment and all M_2- plants lacking the anthocyanin in the vegetative parts should be selected and analyzed for the proanthocyanidins. Normally 10-20 % of the anthocyanin-free plants will also be deficient for proanthocyanidins.

So far several mutants have been selected in more than 100 barley varieties. The mutations can be induced at least 7 independent loci, which all cause blocks in biosynthetic pathway of the proanthocyanidins (3). Pilot maltings and brewings with proanthocyanidin-free mutants resulted in beer with good transparency as well as beer quality as good as the beer produced from the standard varieties. The lack of proanthocyanidins in beer does not change its flavor (2, 3, 7).

One of the mutants (ant 17-148) found in the malting cultivar, Triumph, has been released with the name of Galant. However most of the proanthocyanidin-free mutants have undesirable pleiotropic or additional mutation effects (7). They are unacceptable in agronomical sense. Special efforts are required for the breeding of proanthocyanidin-free lines with high yield and good quality (2, 7).

The purpose of the present study was to evaluate the agronomical characteristics of the proanthocyanidin-free mutants derived from Kaya variety.

MATERIALS and METHODS

The M_2 mutant bulk populations derived from the 15 and 30 krad gamma irradiations of Kaya and Quantum varieties (Hordeum distichum L.) were grown at Tokat and İzmir during the 1985-1986 year.

The plants without anthocyanin in the auricle, awn and chaff were selected. The individual plants selected were grown in the progeny rows in two replications at Tokat during the 1986-1987. A total of 36 mutant progenies derived from Kaya were classified as anthocyanin-less among the progeny rows. There was no mutant selected from Quantum (8-10).

The seed samples of 36 mutant lines mentioned above were sent to the Carlsberg Research Laboratories (in Denmark) in 1989 and they were analyzed for proanthocyanidin by Dr.J.Larsen by employing the Vanillin-HCl technique (1, 7, 11).

The frequency of the proanthocyanidin-free mutants was estimated based on the total M_2 plants and anthocyanin-free mutants.

The following characteristics were measured and expressed as plot mean or as mean of 3 measurements.

Biological yield (g/m)
Grain yield (g/m)
Harvest Index (%)
Number of spikes/m
Number of kernels per spike
Protein content (%)
Heading period (days from May 1)
Length of flag leaf (mm)
Width of flag leaf (mm)
Area of flag leaf (mm^2)
Length of flag leaf sheath (cm)
Plant height (cm)
Spike length (cm)
Spike density (%)

RESULTS

Proanthocyanidin Analysis and Mutant Frequency

It can be seen from Table 1 that a total of 18720 plants were grown in the irradiated Kaya population. Among them 36 plants were found anthocyanin-free. The frequency of visually selected

anthocyanin-free mutants was 1.9×10^{-3}. The number of proanthocyanidin-free mutants was 7 based on the chemical analysis thus giving a frequency of 3.7×10^{-4}. 19 % of the visually selected anthocyanidin-free mutants was also proanthocyanidin-free.

Table 1. Mutants selected as anthocyanin-free and proanthocyanidin-free in the M_2 generation.

Total number of plants	Anthocyanidin-free		Proanthocyanidin-free		
	mutants	frequency	mutants	frequency	%
18720	36	1.9×10^{-3}	7	3.7×10^{-4}	19

The selected proanthocyanidin-free mutants are shown in Table 2. It can be seen from this table that two mutants, M-K-28 and M-K-66 had the kernels all proanthocyanidin-free. The remaining 5 mutant lines had proanthocyanidin-free and proanthocyanidin kernels in mixture.

Table 2. Mutants selected as proanthocyanidin-free based on laboratory analysis.

Mutant	Proanthocyanidin free	Mixtures with proanthocyanidin kernels
M - K - 17	-	+
M - K - 27	-	+
M - K - 28	+	-
M - K - 29	-	+
M - K - 64	-	+
M - K - 66	+	-
M - K - 67	-	+

Quantitative Traits

Some agronomical, morphological and quality characteristics of 2 mutants, 100 % proanthocyanidin-free, and the control population are given in Table 3, Table 4 and Table 5.

It can be seen from Table 3 that the mutant lines were inferior to the control in terms of biological yield, grain yield, spike number and number of kernels per spike. The harvest index and thousand kernels weight of the M-K-66 mutant line was higher than that of the control.

Table 3. Some agronomical characteristics of the mutants and the control population.

Population	Biological yield (g/m)	Grain yield (g/m)	Harvest index (%)	Number of spikes	Kernels per spike	Thousand kernels weight
M - K - 28	154	53	33	50	24	54
M - K - 66	188	76	41	64	25	61
Control	375	132	35	100	29	54

Table 4 shows that the length of flag leaf, the width of flag leaf and the area of flag leaf were higher than those of the control population. Days to heading and length of flag leaf sheath were lower than those of control population. Heading period was shorter in the mutant lines.

Table 4. Flag leaf characteristics, days to heading and protein content of the mutants and the control population.

Population	Days to heading	Length of flag leaf (mm)	Width of flag leaf (mm)	Flag leaf area (mm^2)	Length of flag leaf sheath (cm)	Protein content (%)
M - K - 28	26	103	6,3	413	19,4	12,5
M - K - 66	24	106	5,8	394	21,5	-
Control	43	91	5,4	316	23,8	9,9

Table 5 shows that plant height and spike length in the mutants shorter than those of the control population.

Table 5. Plant height, spike length, spike density and fertility of the proanthocyanidin-free mutants and the control population.

Population	Plant height (cm)	Spike length (cm)	Spike density (%)	Fertility (%)
M - K - 28	76	7,9	31	98
M - K - 66	80	8,9	29	96
Control	83	10,0	29	99

DISCUSSION

Barley mutants without proanthocyanidin could be selected in an irradiated barley population. Although a laboratory assesment of the kernels is a final requirement, mutant plants could easily be selected under the field conditions. Ten to 20 % the anthocyanin-free mutants are also proanthocyanidin-free (2) thus giving a chance for reducing the number of laboratory tests by indirect preselection based on anthocyanidin. In this study the mutant without anthocyanidin first selected in the field and then the selected 36 mutants were controlled in the laboratory. The 7 mutant lines without proanthocyanidin were selected among the 36 mutants of anthocyanidin-free. The 19 % proanthocyanidin-free ratio is close to the upper limit of the frequencies reported earlier (1, 2). This high frequency indicates the success of an indirect selection.

The pleiotropic relationship between anthocyanidin and proanthocyanidin producing loci could be a genetical basis such a success. At least 5 genes working in this way have been postulated. These are ant-13, ant-17, ant-18, ant-21 and ant-22. Some other genes such as ant-1 - ant-12, ant-14, ant-15 and ant-16 do not affect the proanthocyanidin synthesis although they may affect the accumulation of anthocyanidin in the vegetative parts (1). One other gene, ant-19, causses a normal anthocyanin color in the vegetative parts of the plant but blocks the proanthocyanidin accumulation in the grain. Therefore discarded plants should be analysed in the laboratory for an effective selection. Eventhough fast and single seed analysing methods were developed (1, 11), the selection of the ant-19 mutants and the others

which could be similar to ant-19 in the laboratory might be an expensive application in a breeding program. The frequency of the anthocyanin free-proanthocyanidin free pleiotropic mutants was found to be 0.00037 in the present study.

The reaction of genetic material to mutagens could be modified by genotypical and environmental factors which could explain why no mutants selected from Quantum variety. Larsen (2) has indicated a very low chance of selecting desirable mutant plants in certain varieties. In our opinion at least 2 varieties and several mutagens should be applied in a mutation breeding program aiming proanthocyanidin-free barley mutants.

The biological yield, grain yield, spike number, number of kernels were lower in the proanthocyanidin-free mutants but they were higher than the control for thousand kernel weight, flag leaf area, protein content, harvest index and early heading. The individual mutants selected could be classified as macromutants and they result in several changes in plant characteristics (13). This might be the result of the pleiotropic effects as well as condensed mutations in one plant (13, 14). The negative pleiotropic effects of proanthocyanidin-free mutations on the agronomical traits have been reported (2). Therefore the inferiority of the mutants to the control in terms of some agronomical traits could be explained by this phenomenon. The reduction of yield could also be correlated with low tillering, stem thinnes and shrieveled kernels (2, 7). Shrunked kernels could influence grain yield. They also reduces the amount of plump kernels. Shrunkage in kernel causing increase in protein content may be the result of a distruption during the grain filling period as indicated by Larsen et al. (7).

The low grain yield of the mutants in this study is in agreement with the previous results. Reductions in biological yield, number of spikes and number of kernels per spike could result in lower grain yields. The higher thousand kernels weight obtained in this study which contradicts the earlier reports could be due to the longer grain filling period as a result of early heading.

We have not found any report pertinent to earliness associated with proanthocyanidin-free mutations. In general the longer grain filling period of the mutants might be desirable. Previous reports informed that the direct usage of these kinds of mutants in production has been unsuccesfull (2, 5). Therefore the utilization of those mutants in the crossing programs as parents have also been proposed (2, 3, 7).

In order to achieve this aim the mutant gene should be placed in a new genetic background and the negative pleiotropic effect should be eliminated as well as purifing from the deleterious independent mutations. The success of this type of attempts may depend on the suitable combining parents.

CONCLUSIONS

The selection of proanthocyanidin-free mutants from a national barley variety, Kaya, could be considered as a success.

Although the grain yield and some agronomical traits of the mutant lines were found inferior to the control population, a high kernel weight and early heading observed in the mutant lines could be a promising.

ÖZET

PROANTOSİYANİDİNSİZ KAYA ARPA MUTANTLARININ SELEKSİYONU

Biranın berrak görünüşü arzu edilen bir kalite özelliğidir. Arpa danesinden ve şerbetçi otundan gelen bazı polifenoller ile proteinin birleşerek çökelti oluşturması birada bulanıklığa yol açmaktadır. Arpa proantosiyanidinleri bulanıklığa yol açan polifenollerin ana kaynağını oluşturmaktadır. Biranın colloidal stabilitesi ve uzun dönem berraklığı, normal olarak, çökelti oluşturan bileşenlerden bir veya her ikisini etkisiz hale getirmek üzere bazı katkı maddelerinin kullanılmasıyla sağlanmaktadır. Bulanıklık probleminin çözümüne diğer bir yaklaşım, suni mutasyonlar yoluyla proantosiyanidin biyosentezinin bloke edilerek, bu bileşiklerin arpa'danesi üzerinde birikimini önlemektir. Bu çalışmada bu tür mutasyonları seçmek ve bunların agronomic özelliklerini belirlemek amaçlanmıştır. Bu amaçla, tohumları gama ışınları ile muamele edilen Kaya arpa çeşidinin M_2 populasyonları 1985-1986 yıllarında İzmir ve Tokat'ta yetiştirilmiş ve vejetatif kısımlarında antosiyan bulunmayan bitkiler seçilmiştir. Seçilen bitkiler sonraki yıl iki tekerrürlü döl sıraları halinde Tokat'ta test edilmiştir. Döl testi sonucunda, 36 mutant hat antosiyansız olarak gruplanmıştır. Daha sonra bu mutantların tohum örnekleri proantosiyanidin analizi için Carlsberg Araştırma Laboratuvarlarına (Danimarka, 1989) gönderilmiştir. Vanillin-HCl yöntemi uyarınca yapılan analiz sonucunda 7 mutantın aynı zamanda proantosiyanidinsiz olduğu belirlenmiştir.

Antosiyansız mutantların M_2'de yetiştirilen bitki sayısı bazında frekansı $1,9 \times 10^{-3}$; proantosiyanidinsiz mutantların frekansı ise $3,7 \times 10^{-4}$ olarak saptanmıştır.

Antosiyansızlar içinde aynı zamanda proantosiyanidinsiz olanların oranı ise % 19'dur. Antosiyansızlar yoluyla proantosiyanidinsiz mutantların dolaylı olarak seçilmesine dayanan bu yöntemin genetik temeli pleiotropik gen etkisidir.

Mutantların biyolojik verimi, dane verimi, başak sayısı ve dane sayısı kontrolden düşük; bayrak yaprağı alanı, protein miktarı, erkenciliği, hasat indeksi, bin dane ağırlığı ise yüksek bulunmuştur. Mutant hatların genel agronomik preformansının gerilemesi önceden bildirilen sonuçlarla uyum halinde görülmekle birlikte, bin dane ağırlığının artışı, erken başaklanma yoluyla uzayan dane doldurma süresinin dane ağırlığı üzerine olumlu etkisine dayandırılmıştır.

Makro mutasyon kategorisine giren bu tür mutantların doğrudan üretilerek çeşit adayı olma şansları yoktur. Bununla birlikte bu mutantların belirgin olarak erken başaklanması, melezleme programlarında ebeveyn olarak kullanılma şansını artırmaktadır.

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