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

M. İlhan ÇAĞIRGAN* Metin B. YILDIRIM**

SUMMARY

The clear appearance of a beer is a desired quality factor. Colloidal stability and long-lived brilliance 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. M₂- 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 M₂. A total of 38 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 1.9x10⁻³. The number of proanthocyanidin-free mutants was 7 based on the chemical analysis thus giving a frequency of 3.7x10⁻⁴. It was found that 19% of the visually selected anthocyanin-free mutants was also proanthocyanidin-free. The pleiotropic relationship between anthocyanin 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 performance 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 appearance 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 anthocyaninless 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 Vanilillin-HCl technique (I, 7, II).

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 $1.9 \times 10^{-3}$. The number of proanthocyanidin-free mutants was 7 based on the chemical analysis thus giving a frequency of $3.7 \times 10^{-4}$. 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.

<table>
<thead>
<tr>
<th>Total number of plants</th>
<th>Anthocyanidin-free</th>
<th>Proanthocyanidin-free</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mutants</td>
<td>frequency</td>
</tr>
<tr>
<td>18720</td>
<td>36</td>
<td>$1.9 \times 10^{-3}$</td>
</tr>
</tbody>
</table>

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.

<table>
<thead>
<tr>
<th>Mutant</th>
<th>Proanthocyanidin free</th>
<th>Mixtures with proanthocyanidin kernels</th>
</tr>
</thead>
<tbody>
<tr>
<td>M - K - 17</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>M - K - 27</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>M - K - 28</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>M - K - 29</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>M - K - 64</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>M - K - 66</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>M - K - 67</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>

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.

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

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.

<table>
<thead>
<tr>
<th>Population</th>
<th>Days to heading</th>
<th>Length of flag leaf (mm)</th>
<th>Width of flag leaf (mm)</th>
<th>Flag leaf area (mm²)</th>
<th>Length of flag leaf sheath (cm)</th>
<th>Protein content (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>M - K - 28</td>
<td>26</td>
<td>103</td>
<td>6,3</td>
<td>413</td>
<td>19,4</td>
<td>12,5</td>
</tr>
<tr>
<td>M - K - 66</td>
<td>24</td>
<td>106</td>
<td>5,8</td>
<td>394</td>
<td>21,5</td>
<td>-</td>
</tr>
<tr>
<td>Control</td>
<td>43</td>
<td>91</td>
<td>5,4</td>
<td>316</td>
<td>23,8</td>
<td>9,9</td>
</tr>
</tbody>
</table>

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.

<table>
<thead>
<tr>
<th>Population</th>
<th>Plant height (cm)</th>
<th>Spike length (cm)</th>
<th>Spike density (%)</th>
<th>Fertility (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>M - K - 28</td>
<td>76</td>
<td>7.9</td>
<td>31</td>
<td>98</td>
</tr>
<tr>
<td>M - K - 66</td>
<td>80</td>
<td>8.9</td>
<td>29</td>
<td>96</td>
</tr>
<tr>
<td>Control</td>
<td>83</td>
<td>10.0</td>
<td>29</td>
<td>99</td>
</tr>
</tbody>
</table>

DISCUSSION

Barley mutants without proanthocyanidin could be selected in an irradiated barley population. Although a laboratory assessment 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 anthocyanin. In this study the mutant without anthocyanin first selected in the field and then the selected 36 mutants were controlled in the laboratory. The 7 mutant lines without proanthocyanin 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, causes 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 ex-
pensive 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 thinness and shriveled kernels (2, 7). Shrunken kernels could
influence grain yield. They also reduces the amount of plump kernels.
Shrinkage in kernel causing increase in protein content may be the
result of a disruption during the grain filling period as indicated
by Larsen et al. (7).

The low grain yield of the mutants in this study is in agree-
ment 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 unsuccessful (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 purifying 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

PROANTOSOYANIDINİSIZ KAYA ARPA MUTANTLARININ SELEKSIYONU


Antosiyansız mutantların M2 de yetiştirilen bitki sayıısı bazinda frekansı 1,9x10⁻³; proantosiyanidinız mutantların frekansı ise 3,7x10⁻⁴ olarak saptanmıştır.
Antosiyanisler içinde aynı zamanda proantosiyanidinsiz olanların oranı ise % 19'dur. Antosiyanisler yoluya proantosiyanidinsiz mutantların dolayı olarak seçilmesine dayanan bu yöntem genetik temeli pleiotropik gan etkisidir.

Mutantların biyolojik veciri, dene verimi, başak sayası ve dene sayısı kontrolден doğruluk, beyaz yaprakları alani, protein miktarı, erkenliği, hasat indeksi, bitki dengesi, iske feeding ölçümü ve hasat dengesi, bitkinin genel agronomik preformansının gerilemesi üzerindeki deneşine uyum halinde görülmekle birlikte, bitki dengesi ve erkenliği artışı, erken başalaması, yoluya uzayan dene ve doldurma süresinin dene olumu etkisinde dayandırılmıştır.

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

ACKNOWLEDGEMENT

The authors are very thankful to Dr. J. LARSEN, Head of Plant Breeding Unit, Carlsberg Research Laboratories, Copenhagen, Denmark, for proanthocyanidin analysis of the material.

LITERATURE CITED


