# On the DBC protein content and on the amino acid contents in $F_5$ lines of the barley line Hiproly

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**Abstract** Two descendant groups of the barley line Hiproly and the parents, Hja c2661 and Hja c4003, were examined for variations in the DBC protein and amino acid contents. The frequency distributions of the DBC protein content in both descendant groups were unimodal and no effect of one gene was seen, even though the high lysine content of Hiproly is caused by one gene (lys). The means of the DBC protein contents in these $F_5$ generations were 19.1 % and 19.0 %.

Environmental factors have a great effect on the DBC protein content: environmental variances from the total variances were 93.84 % and 41.54 %. In the lines with the highest DBC protein contents there were generally more basic amino acids, phenylalanine and proline, than in the lines with the lowest DBC protein contents. Therefore, it appears that the lines with the highest DBC protein contents contain much albumins and prolamins. Also, the ratios of basic amino acids to proline indicated that the relationships between albumins and prolamins were nearly the same for all the lines. Thus, the high DBC protein content in many lines is really based on high amounts of albumins and prolamins at the same time. The present study indicated that Hiproly may be better than the Risø 1508 mutant of barley in breeding for the yielding capacity, because the lys gene of Hiproly does not seem to decrease the prolamin content.

## 1. Introduction

Although cereals are very important for protein production, the protein is not of high quality because some amino acids are not present in sufficient quantities. Most notable is the small amount of lysine, but also methionine and threonine exist in low amounts in the seed protein of most cereal plants. HAGBERG and KARLSSON (1969), when screening over one thousand barley lines *(Hordeum vulgare* L.), found one barley line Hiproly, which had a high protein and lysine content. The high lysine content of this Ethiopian barley line Hiproly is dependent on a single recessive major gene (lys) and several minor genes (MUNCK et al. 1970, KARLSSON 1972, KARLSSON 1976). HAGBERG and KARLSSON (1969) found the barley line Hiproly by a DBC method, which is based on the dye binding capacity of basic amino acids (lysine, arginine and histidine) and the free amino ends of polypeptide chains. Thus high lysine and high protein lines are screened from other lines (MOSSBERG 1969).

MUNCK et al. (1970) were able to distinguish in the $F_2$ generation of the barley line Hiproly two differing groups: the lines which have a high DBC value and a high lysine content and the lines which have a low DBC value and a low lysine content.

The present study investigates the variation in DBC protein and amino acid contents in the $F_5$ lines of the barley line Hiproly. It has also been possible to study the inheritance of the DBC protein content and the factors on which it depends. The present work is based on a project concerning the breeding of high quality barley varieties at the Hankkija Plant Breeding Institute. Material for the study was obtained from this Institute.

## 2. Material and methods

### 2.1. Material

The material was the harvest of the year 1971. It consisted of the $F_5$ lines of two crossings: Hja c2661 x Hiproly and Hja c4003 x Hiproly, and the parental lines Hja c2661 and Hja c4003. The lines Hja c2661 and c4003 had grown on every tenth plot among the corresponding hybrid lines. Of the $F_5$ lines there were no repetitions on the field. Fertilization was 75 kg/ha N, 44 kg/ha P and 63 kg/ha K (REKUNEN, personal communication). The following varieties and lines of barley were also used as material: cv. Birgitta, cv. Ingrid, cv. Karri, cv. Mari, cv. Mona and Hja c4109.

The hybrid segregants were taken as lines in the $F_3$ generation. Selection against some morphological features was made (REKUNEN, personal communication). It was not possible to get seeds from all lines. From the cross Hja c2661 x Hiproly there were, in this study, 76 lines or 46.7 % of all $F_5$ lines of this crossing. From the cross Hja c4003 x Hiproly there were, in this study, 243 lines which was 85.9 % of all $F_5$ lines.

The barley line Hiproly is a 2-row naked barley with a high protein content (17 %) and a high lysine content (4.1 g lysine/100 g protein) (HAGBERG and KARLSSON 1969). The line Hja c2661 is a 6-row fodder barley, which descends from the crossing cv. Otra x cv. Paavo. The line Hja c4003 is a 2-row barley, which was obtained by radiating the line Hja b7990. The line Hja b7990 is descended from the crossing (cv. Louhi x cv. Opal) x cv. Stallar II (REKUNEN, personal communication).

### 2.2. Determination of DBC protein and amino acid contents

The DBC protein content was analysed by a Pro-Meter Mk II machine (prepared in A/S N. FOSS ELECTRIC, DK 3400 Hillerod, Denmark). Its function is based on the UDYs (1956) method. The machine records the values of proteins as per cent from the sample. Because the DBC protein contents of most lines were more than 17 % (the maximum value which the machine records), samples of 400 mg were used as against samples of 500 mg according to the operation instructions. From three to five repetitions were made from every sample. The water contents of the samples were determined according

to CONGER et al. (1970). The following abbreviation is used: DBCpc = the DBC protein content.

Amino acid analyses were made for 12 lines of Hja c4003 x Hiproly. These were the six lines with the highest and the lowest DBC protein contents. An acid hydrolysis was made at first for 40 mg samples in which tryptophane is destroyed (KOHLER and PALTER 1967). After that the samples were driven by a Hitachi Perkin-Elmer liquid chromatography and a Ligandi method was used (EAKER 1968).

## 2. 3. *Statistical calculation*

Because most samples used to determine the DBC protein content were 400 mg, the values had to be modified to correspond to samples 500 mg in size. For this purpose, a regression model was developed from which it was possible to determine the DBC protein contents in samples of 500 mg as well as in samples of 400 mg. The regression equation was developed by repetitions of the samples of 400 mg (x-variable) and by repetitions of the samples of 500 mg (y-variable). With this regression equation it was possible to estimate the values corresponding to the samples of 500 mg, by using the values derived from the samples of 400 mg. In this equation ($y = bx + a$), x is the value which was determined from a sample of 400 mg, and y corresponds to the value of a sample of 500 mg. b is a regression coefficient and a is a constant.

Differences in the DBC protein content between the crossings and between the lines were tested by the hierarchal analysis of variance (KEMPTHORNE 1969). This calculation was performed in the Computer Centre of the University of Helsinki. Percentages for the components of variance, such as line, cross and repetition, were also calculated.

The environmental and genetic influence on the DBC protein content was estimated according to FALCONER (1967), assuming that the variance of the parental line is an estimate of the environmental variance, and that the variance of descendant lines is an estimate of the total variance.

The percentage content of each amino acid was determined by presuming that there were proportionally the same amounts of tryptophane in each line. The ratio of basic amino acids to proline was calculated for each line (FAVRET et al. 1970) to indicate the proportional amounts of albumins and prolamins.

Otherwise the results were calculated statistically in the usual manner.

## 3. Results

The regression line in Figure 1 shows the relationship between the DBC protein contents and the sample sizes. The correlation coefficient between these values is 0.999***. This method was reliable because the standard error of the estimate of y on x is 0.114. The standard error of the regression coefficient b is 0.008, and the standard error of the constant a is 0.094.

The frequency distributions of the DBC protein contents of the lines in two crossings do not indicate any clear differences between the crossings (see Fig. 2). The lines differ from each other when analysed by the hierarchal
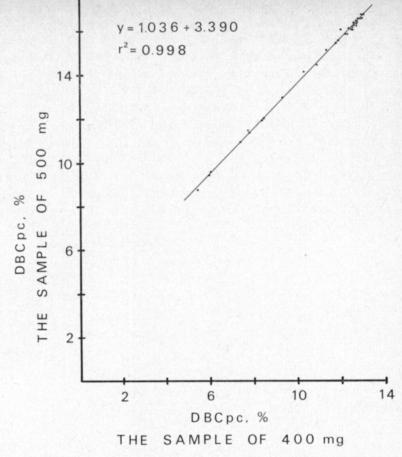
$$y = 1.036 + 3.390$$
$$r^2 = 0.998$$

DBCpc, %

THE SAMPLE OF 500 mg

DBCpc, %

THE SAMPLE OF 400 mg

Fig. 1. A regression line between two sizes of samples.



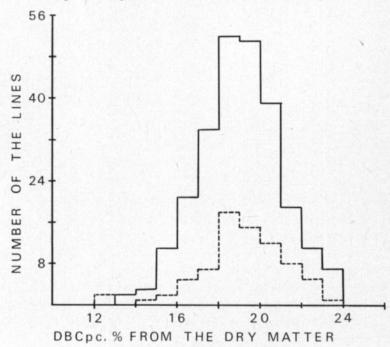NUMBER OF THE LINES

DBCpc, % FROM THE DRY MATTER

Fig. 2. The frequency distributions of $F_5$ lines.
– – – = $F_5$ lines of Hja c2661 x Hiproly.
———— = $F_5$ lines of Hja c4003 x Hiproly.

analysis of variance (F = 3.678***), but between the crossings there is no statistically significant difference. The component of variance caused by variation between the lines is 99.37 % from the total variance. The component of variance caused by crossings is 0 % and the component of variance caused by the variation within the lines is 0.63 % from the total variance.

The means of the DBC protein contents in descendant groups are higher than in the parents (see Table 1). The descendant group of Hja c2661 x Hiproly differs from the parental line with respect to the DBC protein content determined by the analysis of variance (F = 20.220***). In the same way the descendant group of Hja c4003 x Hiproly differs from the line Hja c4003 (F = 23.789***). Between the parental lines there is no statistically significant difference. Environmental factors have greatly influenced the DBC protein content (see Table 1).

Table 1. Means, variances and the components of variance of the DBC protein contents in two experimental groups.

| Experimental group | $\bar{x}$ | $s^2$ | Components of variance | | |
| --- | --- | --- | --- | --- | --- |
| | | | total variance, $V_p$, % | environ- mental variance, $V_e$, % | genetic variance $V_g$, % |
| Hja c2661 x Hiproly: | | | 100.00 | 93.84 | 6.16 |
| Hja c2661 | 16.7 | 4.10 | | | |
| F$_5$ generation of Hja c2661 x Hiproly | 19.1 | 4.37 | | | |
| Hja c4003 x Hiproly: | | | 100.00 | 41.54 | 58.46 |
| Hja c4003 | 17.5 | 1.59 | | | |
| F$_5$ generation of Hja c4003 x Hiproly | 19.0 | 3.82 | | | |

There are differences in some amino acid contents between lines which have high or low DBC protein contents (see Table 2). The lysine contents of protein in the lines with high DBC protein content are 3.54 %, 3.60 %, 3.66 %, 3.89 %, 4.06 % and 4.21 %. The correlation coefficients between the amino acid contents and the DBC protein contents are different (see Table 2). The ratio of the amount of basic amino acids to the amount of proline is nearly the same for every line (see Table 3). There is no statistically significant difference in these relationships between the groups of lines which have the highest or the lowest DBC protein contents.

Table 2. The relative (%) amino acid contents in different groups and the correlation coefficients between the amino acid contents and the DBC protein contents.

| Amino acids | Group 1. low DBCpc n = 6 | | Group 2. high DBCpc n = 6 | | F-test between groups | r |
|---|---|---|---|---|---|---|
| | $\bar{x}$ | s | $\bar{x}$ | s | | |
| Asp | 5.41 | 0.70 | 5.43 | 2.27 | — | +0.01 |
| Glu | 27.91 | 1.52 | 26.19 | 1.55 | — | −0.51 |
| Thr | 3.38 | 0.13 | 3.30 | 0.08 | — | −0.35 |
| Ser | 3.90 | 0.19 | 3.93 | 0.19 | — | +0.11 |
| GluN | — | — | — | — | — | — |
| Pro | 11.14 | 0.62 | 12.31 | 0.84 | * | +0.64* |
| Ala | 4.25 | 0.11 | 4.33 | 0.29 | — | +0.19 |
| Gly | 3.98 | 0.28 | 3.84 | 0.21 | — | −0.26 |
| Val | 5.58 | 0.22 | 5.54 | 0.28 | — | −0.06 |
| Cys | 1.19 | 0.26 | 0.97 | 0.23 | — | −0.42 |
| Met | 1.44 | 0.07 | 1.50 | 0.17 | — | +0.28 |
| Ileu | 4.33 | 0.26 | 4.39 | 0.08 | — | +0.19 |
| Leu | 8.42 | 0.56 | 8.09 | 0.35 | — | −0.35 |
| Tyr | 3.12 | 0.15 | 3.37 | 0.17 | * | +0.68* |
| Phe | 5.48 | 0.18 | 5.75 | 0.20 | * | +0.63* |
| Lys | 3.43 | 0.15 | 3.84 | 0.28 | ** | +0.71** |
| His | 2.12 | 0.15 | 2.18 | 0.07 | — | +0.33 |
| Try | — | — | — | — | — | — |
| Arg | 4.18 | 0.18 | 4.71 | 0.26 | ** | +0.78** |
| Lys + His + Arg | 9.73 | 0.40 | 10.73 | 0.55 | ** | +0.75** |

Significance: — nonsignificant, *P < 0.05, **P < 0.01.

Table 3. The phenylalanine, lysine and proline contents and the amounts of basic amino acids ($\mu$g basic amino acids/g meal) per the amount of proline ($\mu$g proline/g meal) relationships for different lines.

| Line | Type of the line | % phenylalanine | % lysine | % proline | The amount of basic amino acids/the amount of proline |
|---|---|---|---|---|---|
| 1. | low DBCpc | 5.32 | 3.36 | 11.66 | 0.818 |
| 2. | low DBCpc | 5.57 | 3.40 | 11.22 | 0.872 |
| 3. | low DBCpc | 5.41 | 3.19 | 10.14 | 0.890 |
| 4. | low DBCpc | 5.27 | 3.52 | 11.91 | 0.844 |
| 5. | low DBCpc | 5.59 | 3.55 | 11.02 | 0.903 |
| 6. | low DBCpc | 5.74 | 3.58 | 10.91 | 0.920 |
| 7. | high DBCpc | 6.03 | 3.54 | 11.79 | 0.866 |
| 8. | high DBCpc | 5.86 | 3.66 | 12.57 | 0.825 |
| 9. | high DBCpc | 5.47 | 3.98 | 12.69 | 0.854 |
| 10. | high DBCpc | 5.56 | 3.60 | 12.11 | 0.848 |
| 11. | high DBCpc | 5.82 | 4.21 | 11.13 | 1.044 |
| 12. | high DBCpc | 5.75 | 4.06 | 13.59 | 0.815 |

## 4. Discussion

The frequency distributions of the DBC protein contents in the descendant groups are unimodal. The distribution of the lys gene ought to be $3:2:3$ in these descendent groups, because the descendants have been taken as lines in the $F_3$ generation and because barley is highly inbreeding. From the lines 6/8 ought to be homozygotic for this locus. The lys gene increases the lysine content by 30 % compared to typical commercial varieties (MUNCK et al. 1971). In the lines which have the highest DBC protein contents there must be much protein and/or much basic amino asids, because the function of the Pro-Meter Mk II is based on the dye binding capacity. The effect of the lys gene is so great that the frequency distributions ought to be bimodal. Selection in the descendant groups has possibly been able to change the distributions. OLSEN (1974) has also found unimodal distribution of the DBC protein content in the $F_3$ generation of the barley line Hiproly and he has not been able to see any effect of one gene in that distribution.

The correlation coefficient ($r = 0.71$) between the DBC protein content and the lysine content is low compared to the correlation coefficient ($r = 0.93$) between the lysine content and the amount of bound dye obtained by HAG-BERG and KARLSSON (1969). The mean lysine content ($\bar{x} = 3.84$ %) in the lines which have the highest DBC protein contents is only slightly higher than the mean lysine content ($\bar{x} = 3.44$ %) in the lines which have the lowest DBC protein contents. This explains the unimodality of the frequency distributions.

The lysine content of protein was estimated to be over 4 % in two barley lines and nearly 4 % in one barley line. In the barley line Hiproly and in the hybrid lines, which have been homozygotic for the lys gene, there has always been lysine more than 3.9 % and nearly always over 4 %, while in usual commerical barley varieties and in not hily (high lysine) segregants of the line Hiproly there has been $3.25-3.90$ % lysine depending on the protein content (MUNCK et al. 1969, HAGBERG et al. 1970, MUNCK 1970, MUNCK et al. 1970, MUNCK et al. 1971, MUNCK 1972 a, MUNCK 1972 b). Obviously only those lines in which the lysine content is over 4 % can be homozygotic for the lys gene. However, the lysine content of every line with a high DBC protein content, is above average because there is usually a high negative correlation between the lysine content and the protein content (HAGBERG and KARLSSON 1969). Evidently all lines which have the highest DBC protein content are not homozygotic for the lys gene, but are mixtures consisting of many lines homozygotic for the lys or wild allele at this locus.

The correlation coefficient between all basic amino acids (Arg + Lys + His) and the DBC protein is higher than the correlation coefficient between lysine and DBC protein. This is consistent with the result of MOSSBERG (1969). The highest correlation coefficient is, however, between the arginine content and the DBC protein content.

The DBC protein contents of the parent lines are also very high. N and P fertilization may have brought about this result. It is apparent that environmental factors have a great effect on the DBC protein content since there is a

great variation in the DBC protein content in the parental lines. There is more phenylalanine in lines which have the highest DBC protein contents. This means that there must be much prolamins in those lines (see HARRIS 1962). This may be due to N fertilization, which increases the proportional amount of prolamins in barley seed proteins (ANDERSEN and KØIE 1975).

The amounts of basic amino acids — proline relationships also indicate that the relationships between albumins and prolamins are nearly the same for all 12 lines. FAVRET et al. (1970) believe that this relationship expresses the proportional amounts of these protein components. If the value is 1, the relationship is normal, if it is > 1, there are more albumins rich in lysine, and if it is < 1, there are more prolamins poor in lysine. In the lines which have the highest contents of DBC protein, there must be much albumins and prolamins present simultaneously.

The high phenylalanine and proline contents of the high DBC protein lines in the present study indicate that the lys gene does not decrease the prolamin content. BERDAHL and BHATTY (1977) have found that Hiproly and a normal lysine barley line and the high lysine plump segregants of Hiproly, have the same prolamin content, but the shrunken segregants of Hiproly have a slightly lower prolamin content than Hiproly. In contrast to Hiproly, Risø 1508, the other high lysine mutant of barley (DOLL 1973, MUENCH et al. 1976), has only one-third of the normal prolamin content (INGVERSEN et al. 1973).

The environmental influence on the protein content has proved very considerable even in this one field experiment. This was also noted by FAVRET et al. (1970), who considered the absolute amount of protein of one seed (N/seed) to be more stable than the protein content, expressed as per cent from the dry matter. Environmental factors affect the weight and volume of a seed. Furthermore, the weight and the volume of a seed are negatively correlated with the protein content, while the absolute values are less variable. When proteins are expressed as per cent values from the dry matter, it is difficult to find the amount of variation which is caused by genetic factors. A possibility for decreasing the variation in the protein content caused by environmental factors lies in the use of powerful N fertilization. Increased N fertilization raises the protein content and simultaneously decreases variation in the protein content caused by environmental factors. Thus the genetic variance of the protein content increases in relation to the total variance (ULONSKA et al. 1975). Selection studies performed under powerful N fertilization can of course be criticized for economical reasons.

The greatest difficulty in breeding high lysine barley varieties lies in maintaining the yielding capacity at a high enough level. It seems that inactivation of prolamin synthesis impairs the accumulation of carbohydrates in the endosperm (DOLL 1977) thus lowering the yielding capacity. Hiproly can be better than Risø 1508 in breeding for yielding capacity since it has a high prolamin content. In Risø 1508 the high lysine content is the result of one gene that blocks the synthesis of several protein bands with high molecular weight in the prolamin fraction (BRANDT 1976, KØIE et al. 1976). In Hiproly INGVERSEN and KØIE (1971) have found an extra lysine rich band in the salt-soluble protein fraction.

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SELOSTUS

## DBC-proteiinipitoisuudesta ja aminohappojen määristä Hiproly ohran $F_5$ linjoissa

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Tutkimusaineisto käsitti Hja c2661 x Hiproly ja Hja c4003 x Hiproly risteytyksen $F_5$ sukupolven linjat sekä kantavanhemmat Hja c2661 linjan ja Hja c4003 linjan. DBC-proteiini-pitoisuus määritettiin Pro-Meter Mk II laitteistolla. Aminohappoanalyysi suoritettiin Hja c4003 x Hiproly risteytyksen 12 linjalle: kuudelle DBC-proteiinipitoisuudeltaan korkeimmalle ja alhaisimmalle linjalle.

DBC-proteiinipitoisuuksien määrityksessä kehitettiin regressiomalli, jotta voitiin käyttää normaalia pienempiä näytteitä, koska proteiinipitoisuudet olivat yleensä korkeampia kuin laitteiston maksimilukema. DBC-proteiinipitoisuuksien frekvenssijakautumat olivat $F_5$ suku-polvissa yksihuippuiset, eikä niissä voitu havaita Hiproly ohran lys -geenin vaikutusta.

Hja c2661 x Hiproly risteytyksen jälkeläistön DBC-proteiinipitoisuuden keskiarvo oli 19.1 %, Hja c4003 x Hiproly risteytyksen jälkeläistön 19.0 %, Hja c2661 linjan 16.7 % ja

Hja c4003 linjan keskiarvo oli 17.5 %. Ympäristötekijöillä oli suuri vaikutus DBC-proteiini-pitoisuuteen: ympäristötekijöiden aiheuttama varianssin komponentti kokonaisvarianssista oli 93.84 % ja 41.54 % näissä kahdessa koeryhmässä.

DBC-proteiinipitoisuudeltaan korkeatasoisissa linjoissa oli enemmän proliinia, tyrosiinia, fenylalaniinia, lysiiniä ja arginiinia kuin linjoissa, joiden DBC-proteiinipitoisuus oli alhainen. Kahdessa linjassa oli lysiiniä yli 4 %, millä perusteella ne saattoivat olla lys -geenin suhteen homotsygoottisia. Emäksisten aminohappojen määrä per proliinin määrä suhteet olivat kaikille linjoille lähes samansuuruiset. DBC-proteiinipitoisuudeltaan eritasoisten linjojen välillä ei ollut tässä suhteessa eroa. Korkeat DBC-proteiinipitoisuudet johtuivat aminohappoanalyysien perusteella samanaikaisesta albumiinien ja prolamiinien korkeasta määrästä. Lys -geeni ei alenna prolamiinien määrää. Tällä perusteella Hiproly ohra soveltuu paremmin kuin Risø 1508 mutantti korkeasatoisten ja korkeaa lysiinipitoisuutta olevien lajikkeiden jalostukseen.