THE EFFECT OF REDUCING CONDITIONS ON HELIANTHININ PROTEIN PROFILE OF SUNFLOWER (HELIANTHUS ANNUUS L.)

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ABSTRACT

The SDS-PAGE analyses for 11S globulin helianthinin in two sunflower hybrids were made under reducing and non-reducing conditions. The results revealed that under reducing conditions, the observed number of protein bands was more, the resolution of protein bands was better and also the mobility of protein bands was faster, as compared to non-reducing conditions.

SDS-PAGE (Sodium dodecyl sulphate-polyacrylamide gel electrophoresis analysis) for studying its 11S globulin (helianthinin) protein profile pattern is usually done for identification of genotypes under reducing conditions (Reymond et al., 1991; Anisimova et al., 1993). The helianthinin has reportedly low amount of sulphur containing amino acid (Derbyshire et al., 1976). Hence, reducing conditions supposedly should have little impact on the expression and clarity of protein based patterns in SDS-PAGE analyses. In the present study we observed the effects of reducing and non-reducing conditions on the expression, clarity and polymorphism of protein bands.

The SDS-PAGE analysis on two sunflower hybrids, namely, KBSH-1 and PAC 304 was made following the procedure described by Laemmli (1970). Proteins were extracted following the procedure of Dalgalarrondo et al. (1984) with some modifications. Individual decoat seeds were defatted in n-hexane. Salt soluble proteins from sunflower meal of each defatted seed were extracted in 0.3 ml of 0.02 M borate buffer, pH 8.1, containing 10% NaCl and 1m M PMSF, separately placed at 4°C for 30 minutes. The mixture was centrifuged for 15 minutes at 10,000 rpm. The supernatant was collected and separated into albumin and globulin by adjusting pH at 4.8. The extract was dialysed overnight against 0.03 M sodium acetate buffer, pH 4.8; and centrifuged for 3 minutes at 15,000 rpm. The globulin pellet was resuspended in distilled water and solubilized by increasing pH to 8.0 with NaOH. The solubilized globulin fraction was then dialysed against distilled water and kept at 4°C for SDS-PAGE analysis. Using procedure described by Laemmli (1940) with a modification in concentration of separating gel buffer (1 m), SDS-PAGE was performed on 1mm thick gel slab. For electrophoresis under reducing conditions 2 per cent 2-mercaptoethanol in sample buffer was added. The sample buffer per se (without addition of 2-mercaptoethanol) provided non-reducing conditions. Equal quantities of protein sample and sample buffer were mixed and incubated at 100°C for 5 minutes in water bath. Electrophoresis was carried out initially at 90 V through stacking gel (6% acrylamide) and later on at 110 V through separating gel (12.5% acrylamide). The gels were stained overnight in 0.25 per cent Comassie BBR-250 in 100 ml methanol and 40 ml acetic acid and destained by 3% NaCl solution. The gels were analysed and relative mobility of protein bands were calculated as follows:

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\text{Relative mobility (Rm)} = \frac{\text{Distance between origin and migrating band}}{\text{Distance between origin and tracking dye}}
\]
### Table 1. Effect of reducing and non-reducing conditions on total number, relative mobility (Rm values) and expression of protein bands in sunflower hybrids

<table>
<thead>
<tr>
<th>Name of hybrids</th>
<th>Non reducing conditions</th>
<th>Reducing conditions</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Total no. of protein bands</td>
<td>Rm value</td>
</tr>
<tr>
<td>KBSH-1</td>
<td>Zone I 0.015, 0.060, 0.121, 0.152, 0.242, Zone II 0.439, 0.470</td>
<td>12</td>
</tr>
<tr>
<td>PAC 304</td>
<td>Zone I 0.150, 0.060, 0.136, 0.152, 0.220, 0.242, 0.295</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>Zone II 0.364, 0.470; Zone III 0.735</td>
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</table>

The results obtained for the two sunflower hybrids are presented in Table 1. The results obtained clearly revealed that the total number of observed protein bands were greater in number under reducing conditions than in non-reducing conditions that is, 19 and 14 for hybrids KBSH-1 and PAC304, respectively as compared to non-reducing conditions where these were 12 and 11, respectively. The Rm values were also greater under the reducing condition as compared to non-reducing conditions. Hybrid KBSH-1 had Rm value 0.735 (of last protein bands) in non-reducing conditions which was less than the Rm value 0.780 (of last protein bands) under reducing conditions. In addition, there were several other protein bands which under reducing conditions had greater Rm value as compared to non-reducing condition in three different zones (Table 1). Similarly, the hybrid PAC 304 had greater Rm values under reducing conditions ranging from 0.015 to 0.636 as compared to a range 0.015 to 0.470 (value of zone I and II included) under non-reducing conditions. Thus the occurrence of these changes in the presence of 2-mercaptoethanol are due to the separation of helianthinin subunits into low molecular weight polypeptides which resulted due to breaking of interpolypeptide disulphide bonds. The separation gave the better expression and faster mobility of protein bands and also increased the total number of protein bands. Some previous workers, however, have reported the slow mobility and no change in number of protein bands under reducing condition, which may be due to the unfolding of polypeptides (Allore and Barber, 1984; Anisimova, et al., 1995).

In hybrid PAC 304 under reducing conditions we also observed the slow relative
mobility of protein bands which were visually appearing of similar thickness as compared to hybrid KBSH-1. The Rm value of a band was 0.242 in hybrid PAC 304 which was obviously less than the Rm value 0.258 of KBSH-1 (Table 1). Likewise, the movement of three other bands with Rm value 0.470; 0.492 and 0.515 of KBSH-1 was comparatively faster than the three bands with Rm value 0.462, 0.485 and 0.500 of PAC 304, respectively (Table 1). Although a number of factors can effect the relative mobility of protein bands during electrophoresis but presence of higher amount of sulphur containing amino acid within polypeptides in hybrid PAC 304 than KBSH-1 may be responsible for slow mobility of bands due to unfolding of protein polypeptides (Laemmli, 1970). Thus we concluded from the above findings that reducing conditions are essential for polymorphism, better resolution expression and clarity of protein bands during SDS-PAGE analysis.

ACKNOWLEDGEMENT
The authors are thankful to Dr. G.K. Garg and Dr. Anil Kumar of Department of Molecular Biology & Genetic Engineering, GBPUA&T, Pantnagar for laboratory facilities for carrying out the present investigations.

REFERENCES