Chemotaxonomic studies in *Cynodon dactylon* (L.) Pers. complex

II. Flavonoid patterns and ascorbic acid content

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Abstract. Analysis of flavonoid patterns in the diploid, triploid and tetraploid forms of *Cynodon dactylon* revealed them to be cytotype-specific. The triploid forms showed a mixture of spots from the diploid and the tetraploid and in addition exhibited some novel spots. Further, these patterns indicated allopolyploid nature of the tetraploids. The leaves of triploid forms contained almost double the quantity of ascorbic acid present in the diploid and tetraploid forms.

Keywords. *Cynodon dactylon* (L.) Pers.; chemotaxonomy; flavonoids; ascorbic acid.

1. Introduction

This paper is in continuation of the work reported earlier (Sachdeva and Bhatia 1979) and presents information on the flavonoid patterns and ascorbic acid content in respect of two forms of diploids (*D₁*, *D₂*; *n* = 9), two forms of triploids (*TR₁*, *TR₂*; 2*n* = 27) and a form of tetraploid (*TT*; 2*n* = 36). In flavonoid analyses, though the identification of compounds is significant in understanding evolution, a useful information can also be gathered from unidentified chromatographic spots (Grant 1968; Harborne 1975; Dass *et al* 1976).

2. Materials and methods

The leaf material used for the present study was obtained from the same plants on which our previous findings were based (Sachdeva and Bhatia 1979). The method of Mabry *et al* (1970) was employed to study the flavonoid patterns. Ascorbic acid content was determined by the method of Aberg (1958).

3. Observations

Two-dimensional paper chromatographic patterns of flavonoids of diploids (*D₁*, *D₂*), triploids (*TR₁*, *TR₂*) and tetraploid (*TT*) forms are shown in figure 1. The
D₁ form showed 13 spots. The D₂ form exhibited all the spots of the D₁ form except the spot D-8. The TR₁ and TR₂ forms revealed 17 similar spots while in the tetraploid form (TT) 13 spots were noticed. The chromatograms were

Figure 1. Two-dimensional paper chromatographic patterns of flavonoids of diploids (D₁, D₂), triploids (TR₁, TR₂) and tetraploid (TT) forms. Explanation in text.
observed under visible light, ammonia, UV light, UV light/ammonia and Rf values of different spots were determined in BAW and HOAC. On this basis, various spots were tentatively identified for different groups of flavonoids using criteria given by Geissman (1962), Harborne (1967) and Mabry et al (1970). In diploids \((D_1, D_2)\) all the spots corresponded to flavones except 11 and 13 which were isoflavonone/flavonone. In triploids \((TR_1, TR_2)\) spot number 6 corresponded to anthocyanin, and numbers 9 and 12 to isoflavonone/flavonone, 15 and 16 to isoflavone/flavone and rest to flavones. Similarly in tetraploid \((TT)\), spot number 8 corresponded to anthocyanin, 9 and 11 to isoflavonone/flavonone, 13 to isoflavone/flavone and rest to the flavones.

Eight spots (fully darkened) were common to all cytotypes. Some spots characteristic of each cytotype were also observed. The spots D–8 and TT–1 were characteristic of \(D_2\) and TT forms respectively, whereas, spots TR–10, 11, 15, 17 were confined to \(TR_1\) and \(TR_2\) forms only. The tetraploid form lacked spots corresponding to D–9 and 13 of diploids though these were represented in the triploid forms \((TR–7, 16)\). Further triploids showed three spots \((TR–6, 9, 14)\) corresponding to TT–8, 9, 13 of the tetraploid. However, these were absent in the diploids.

The diploid and tetraploid forms possessed almost the same concentration of ascorbic acid while its amount was approximately double in the triploid forms (table 1).

4. Discussion

The pattern of flavonoids in both the diploids was almost similar except that the \(D_1\) form possessed an extra spot, D–8. This appears to be the effect on some biochemical pathway of a number of translocations which could be observed at \(M_1\) of meiosis in the \(D_4\) form (cf. Sachdeva and Bhatia 1979). The tetraploid form was conspicuous by the occurrence of one spot, TT–1 (\(\alpha\) flavone). The triploid forms exhibited a mixture of spots from the diploid and the tetraploid and in addi-

<table>
<thead>
<tr>
<th>Cytotype</th>
<th>Conc. of ascorbic acid mg/100 gms of the tissue</th>
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<tbody>
<tr>
<td>Diploid ((D_1))</td>
<td>9.970</td>
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<tr>
<td>Diploid ((D_2))</td>
<td>9.850</td>
</tr>
<tr>
<td>Triploid ((TR_1))</td>
<td>20.260</td>
</tr>
<tr>
<td>Triploid ((TR_2))</td>
<td>20.050</td>
</tr>
<tr>
<td>Tetraploid ((TT))</td>
<td>10.170</td>
</tr>
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tion possessed some novel spots. Chromatographic studies of leaf flavonoids both in natural and artificial hybrids have shown that inheritance is normally additive, though occasionally some parental constituents are missing or some additional hybrid compounds are present (Harborne 1975). Though no explanation for such a situation has been offered, it appears, that it might be an effect of the accumulation of certain genetic changes during the course of evolution. In the present case, triploids probably were established a long time ago and in them genetic changes responsible for the production of novel flavonoids appeared in the course of evolution in response to higher fitness for disease resistance and insect attack. Smith and Levin (1963) and Levin (1968) demonstrated that the doubling of the genomes does not normally affect flavonoid profile. Presently, tetraploids did possess some spots revealed by diploids but in addition also exhibited some different additional spots establishing, thereby, their allopolyploid origin and, thus, conforming to the conclusions of Tripathi et al (1977) who had earlier established their allopolyploid nature on cytogenetical grounds.

The present data when taken in conjunction with that reported earlier (Sachdeva and Bhatia 1979) reveals that a good amount of chemical variability is exhibited by the three cytotypes. Further, this species is outbreeding, reproduces extensively by vegetative means and has male sterile triploid individuals. In view of these facts, there appear extremely good chances of exploiting the observed chemical variability in different cytotypes to the further improvement of this species by selective hybridisation.

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