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Chemotaxonomic studies in *Cynodon dactylon* (L.) Pers. complex. I. Data on free amino acids, soluble sugars, acid invertase activity and total proteins

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Abstract. Three cytotypes ($2n = 18$; $2n = 27$; $2n = 36$) of *Cynodon dactylon* were found to occur in Punjab plains. Evidences from biochemical analyses were sought to distinguish these otherwise morphologically indistinguishable cytotypes. It was observed that the presence or absence of β-alanine and threonine in the flag leaves could be effectively used as a marker. Tetraploids had highest acid invertase activity which was accompanied with higher concentrations of hexoses and always with reduced concentration of sucrose. Acid invertase activity was least in triploids. Tetraploids were richer in protein content than triploids and had almost double the quantity present in diploids.

Keywords. *Cynodon dactylon*; chemotaxonomy; amino acids; soluble sugars; acid invertase; proteins.

1. Introduction

Cynodon dactylon, popularly known as Bermuda grass, is highly valued as a fodder and a lawn grass. It is widely distributed throughout the tropical and sub-tropical regions of the world. In a previous communication (Tripathy *et al* 1977), the polytypic nature of the species was demonstrated; it comprised several biotypes. Such a polymorphism was attributed to polyploidy within the species, presence of cross- and self-pollination and to an efficient mode of sexual and vegetative reproduction. However, the various cytotypes did not display marked morphological differences. An extensive survey of Punjab plains revealed the occurrence of three cytotypes ($2n = 18$; $2n = 27$; $2n = 36$). The aim of the present work was to seek the evidences from the biochemical analyses to evaluate if these cytotypes were biological units of distinct taxonomic rank. Work on flavonoids, protein profiles, and some of the enzymes is in an advanced stage and will be published subsequently.

2. Materials and methods

The material presently studied was collected from the plains of Punjab and cultured under uniform nursery conditions in the University Botanical Garden.

The samples for chemical analyses were collected from plants where inflorescences emerged out. From such culms, as soon as the inflorescences just opened out, flag leaves were always sampled. Free amino acids and soluble sugars were determined by the methods of Yemm and Cocking (1955) and Shallenberger and Moores (1957), respectively. The method of Pollock and Lloyd (1977) was employed for the extraction and assay of acid invertase. Leaves collected at the same developmental stage were stored at 4° C for 12 hr and extracted in 0·067 M phosphate buffer pH 7·0 in a pre-chilled pestle and mortor and were centrifuged at 15000 × g for 15 min. Supernatant constituted crude enzyme extract. All these steps were carried out at 4° C. The enzyme reaction mixture consisted of 0·2 ml of enzyme extract and 0·8 ml of substrate consisting of 25 mM sucrose in acetate buffer pH 4·8. The reaction was started with the addition of enzyme extract and was allowed to proceed for 1 hr at 37° C. Then 1 ml of DNSA reagent mixture was added to the reaction mixture and boiled for 10 min. Boiled reaction mixture was diluted to 10 ml with distilled water and optical density was noted at 560 nm. The amount of glucose and fructose formed was calculated from standard curve prepared by using glucose.

Total protein content was determined by the method of Lowry *et al* (1951) using Folin ciocalteu-phenol reagent.

3. Results and discussion

Presently, two types each of diploids (D_1, D_2), triploids (TR_1, TR_2) and a form of tetraploid (TT) were investigated. The two forms of diploids were morphologically nearly similar but differed in their cytological behaviour. While D_1 showed persistently 9IIs at prometaphase, D_2 exhibited various degrees of secondary associations presumably resulting from a varying number of translocations. However, A_I and A_{II} in the latter form were perfectly normal resulting in well-filled and stained pollen grains. On the contrary, the two forms of triploids (TR_1 and TR_2) were cytologically similar but one form (TR_2) was more robust and preferred both sunny and shady localities. Even when grown for more than two years under uniform nursery conditions TR_2 retained its distinct robust nature. The tetraploid form (TT) was as robust as TR_2 form. Thus, in the field and even when grown under uniform nursery conditions, it was not usually possible to distinguish the tetraploid form from TR_2 individuals and likewise the diploids from TR_1 form of the triploid. A detailed study of the spikelet in different cytotypes also did not reveal any diagnostic morphological differences. Though in a previous communication (Tripathy *et al* 1977) emphasis was laid on the outline of leaf margin as a distinguishing character for identifying different cytotypes, the present study revealed this character also to be unreliable since it appeared to be under polygenic control. However, the size and fertility of pollen grains could be used for ascertaining the ploidy level. The size of the fertile pollen in triploids and tetraploids was much larger compared to diploids. The higher percentage of pollen infertility distinguished triploids (88%) from tetraploids (4%).

It is quite apparent from what has been appended above that morphological data do not provide any unequivocal diagnostic features. Analysis of free amino acids revealed interesting results (table 1). Diploids (especially D_1 form) had a

Table 1. Free amino acids (mg/g fresh leaf wt) in different cytotypes of *Cynodon dactylon.*

Amino acid	Diploid (D_1)	Diploid (D_2)	Triploid (TR_1)	Triploid (TR_2)	Tetraploid (TT)
Leucine + isoleucine	1·706	1·406	1·975	2·650	2·187
γ-aminobutyric acid	1·773	1·620	0·342	0·345	0·345
Tyrosine	0·109	0·125	0·206	0·187	0·206
Glycine + serine	0·875	0·845	4·756	5·000	6·005
Aspartic acid	0·206	0·274	0·562	1·100	1·800
Glutamic acid	0·156	0·187	2·352	2·200	1·800
Cystein + cystine	..	..	2·075	2·500	0·275
Asparagine	0·095	0·156	1·562	1·562	0·140
Lysine	0·125	0·125	0·562	0·345	0·109
Arginine	0·175	0·187	0·850	0·575	0·625
Glutamine	0·095	0·109	1·562	1·562	5·625
α-Alanine	0·342	0·345	5·850	6·406	0·344
Proline	0·165	0·118	0·532	0·798	0·162
Valine + methionine	0·345	0·342	3·280	3·502	1·050
β-alanine	5·000	2·650	2·075	2·105	..
Threonine	3·438	2·650	..	..	..

very high concentration of β-alanine and threonine, the tetraploid form was conspicuous by their total absence. In the triploids (TR_1, TR_2) though β-alanine was present but threonine was always found to be absent. Thus, the presence or absence of β-alanine and threonine could be effectively used in distinguishing diploid, triploid and tetraploid cytotypes. In addition, the three cytotypes also differed in their amino acids level. For instance, the diploids were rich in γ-amino-butyric acid whereas the triploids had high levels of glutamic acid, cysteine and cystine, asparagine, α-alanine, proline and valine and methionine. In the tetraploids high concentrations of aspartic acid and glutamine were observed. A perusal of the pertinent literature reveals that amino acids have been employed less frequently in plant classifications compared to certain other groups of chemical compounds. However, whenever such studies were made, patterns fitting the traditional taxonomies (Bell 1962; Bell and Tirimanna 1963; Dunnill and Fowden 1965; Seneviratne and Fowden 1968; Peterson and Parris 1970) as well as discordant with them (Reddi and Phipps 1972) have been observed. Though the study of free amino acids was considered as a " taxonomic noise " by Reddi and Phipps (1972), the present study along with those of Reuter (1957), Taira (1966), Byers (1971) and Watson and Creaser (1975) suggests ample usefulness of this marker in taxonomy. However the type of characters which would prove more helpful in systematic studies actually depends upon the groups with which a systematist is concerned. In some groups morphological data alone may provide diagnostic clues while in other cases cytological, anatomical, chemical or palynological data may prove more discriminatory.

Another interesting point which emerges from the present studies pertains to proline. There is evidence to suggest that if proline content of the leaves was

raised by artificial means the resistance to desiccation also increases (Tyankova 1966; Hubac 1967). Goas (1965) reported that proline was dominant free amino acid in some halophytes which points out that its accumulation probably serves some useful purpose in protecting the plant from stress of reduced water levels. In the present case, high levels of proline in the triploids appear to be responsible for their prevalence in a variety of habitats. TR_2 form with more of proline occurs on much divergent habitats than the TR_1 form. Both diploids and tetraploids on the other hand, are quite restricted in this sense.

When fresh leaves at the same developmental stage were analysed for glucose, fructose and sucrose fractions, in the tetraploid there was absence of sucrose while triploids revealed extremely high levels of this disaccharide (cf. table 2). The tetraploids were much richer in glucose and fructose. Diploids always exhibited very low concentrations of sucrose. Further, diploids were comparatively richer in glucose fraction though they were almost as rich in fructose content as were the triploids. The mere absence of sucrose in tetraploids and their extremely high levels in triploids single out these taxa as being peculiarly different genetically. Acid invertase is known to catalyse the hydrolysis of sucrose to hexoses and also plays a key role in growth by controlling sucrose storage and utilisation (Ricardo and Rees 1970; Maclachlan *et al* 1970; Shukla *et al* 1973), the activity of this enzyme was also assayed. As expected, tetraploids had highest activity whereas it was least in the triploids (table 2). In fact, higher invertase activity was accompanied with higher concentration of hexoses but always with reduced concentration of sucrose. The present findings agree with those of Pressey and Shaw (1966) and Lyne and Rees (1971). Curiously enough, Manning and Maw (1975) did not observe invertase-sugar inter-relationship in laminar tissues from *Lycopersicum esculentum*. With respect to rate of growth, diploids and tetraploids were comparatively fast growing than the triploids. Analysis of total proteins in the different taxa revealed that tetraploids were richer than the triploids and possessed almost double the quantity present in diploids (table 2).

Table 2. Acid invertase activity, level of soluble sugars and protein content in leaves of *Cynodon dactylon* cytotypes.

Cytotype	Invertase activity (μg Glucose + Fructose produced/mg of protein/hr)	Soluble sugars (mg/g of Fr. wt.)			Protein content mg/g of Fr. wt.
		Sucrose	Glucose	Fructose	
Diploid (D_1)	250	0·500	5·210	3·356	1·440
Diploid (D_2)	167	1·512	5·800	3·451	1·480
Triploid (TR_1)	112	11·523	4·380	3·807	2·080
Triploid (TR_2)	146	9·510	4·250	3·680	2·240
Tetraploid (TT)	512	..	8·720	10·155	2·890

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