'GLYZAGLABRIN', A NEW ISOFLAVONE FROM *GLYCYRRHIZA GLABRA*

DURING the course of our investigation on the phenolic components of *G. glabra* roots we earlier reported the unusual occurrence of 2-methylisoflavones[1,2] and liqcoumarin[3]. In the present communication we report the occurrence of a new isoflavone, herein named, glyzaglabrin in *G. glabra* roots.

The extraction and fractionation procedures have been already reported[1]. The ether insoluble fraction was chromatographed over silica gel column. The ethylacetate eluate on preparative T.L.C. purification using benzene gave glyzaglabrin, m.p. 224–5°, gave green colour with alc. $FeCl_3$, the Na-Hg/HCl and gallic acid/H_2SO_4 tests for isoflavones and $-O-CH_2-O-$ groups. λ_{max}^{MeOH} (log ε) 225 (4·10), 295 (3·44), + Al Cl_3; 270, 310 nm, + NaOAc; 278, 310 nm. ν_{max}^{KBr} 3400 (OH), 1640 (chelated >C=O), 1550, 1410, 1250, 1240, 1190, 1180, 1070, 1045, 1015, 935 (–O–CH_2–O–), *MS*: m/e 298 (M^+, 100%), indicated that compound to have 16 carbon atoms, 297 ($M^+ - 1$, 80%), 286 ($M^+ - CH_2 + 2H$, 50%), 270 ($M^+ - CO$, 20%) 162 (RDA fragment with ring B, 55%), 136 (RDA fragment with ring A, 16·5%), 150 ($162 - CH_2 + 2H$, 25%), 118 ($136 - H_2O$, 52·5%). On acetylation (Ac_2O/C_5H_5N, 24 hours) at room temperature it yielded an acetate, m.p. 218–9°. *P.M.R.* (δ, $CDCl_3$, TMS as internal standard); 2·75 (6 H, s, 2 × $OCOCH_3$), 5·75 (2H, s, 3′, 4′-O-CH_2-O-), 6·55 (2H, d, J = 8·5 Hz, $C_{5'}$ – H and $C_{6'}$ – H), 6·90 (1H, d, J = 10 Hz, C_6 — H), 7·10 (1H, s, C_8-H), 7·50 (1H, s, C_2 – H), 8·00 (1H, d, J = 10 Hz, C_5 – H).

The spectral data showed the presence of two hydroxyls and a methylenedioxy group in the isoflavone skeleton. Its U.V. spectrum showed bathochromic shifts with NaOAc and $AlCl_3$[4] characteristic of a free hydroxyl as well as a chelated hydroxyl functions in glyzaglabrin. In addition green ferric reaction indicated the possibility of an hydroxyl being chelated with carbonyl group which could be considered at C_8 position. In the P.M.R. the signals at δ 6·90 and 8·00 were assigned to the ortho-coupled aromatic protons present at C_6 and C_5 positions respectively and two singlets at δ 7·10 and 7·50 were assigned for C_8 and C_3 protons respectively[5]. Thus, one hydroxyl is placed at C_7 position in glyzaglabrin, the remaining hydroxyl and methylenedioxy groups were placed at $C_{2'}$, $C_{3'}$ and $C_{4'}$ position respectively. The doublet at δ 6·55 was assigned for two aromatic protons at $C_{5'}$ and $C_{6'}$. Thus, glyzaglabrin was assigned the structure 7,2′-dihydroxy, 3′,4′-methylenedioxyisoflavone. The M.S. also supported this structure as m/e 162 for RDA fragment with ring B and m/e 136 of ring A and the loss of $-CH_2$ group (m/e 150) from the ring B of the RDA cleavage showed that one hydroxyl and a methylenedioxy groups were present at ring B.

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INFLUENCE OF NITROGEN NUTRITION AND SHEATH ROT DISEASE ON SUGAR CONTENT OF TWO PADDY VARIETIES

Introduction

IN India sheath rot disease of rice caused by *Acrocylindrium oryzae* is assuming serious proportions. Yield losses up to 85% had been recorded by Prabakaran *et al.*[12] due to *A. oryzae* infection in Tamil Nadu. Mohan and Subramanian[8] reported 57% damage due to the disease. It was found that increased nitrogen content led to the susceptibility of the plants (Chien and Chin[3], Mohan and Subramanian[8]). With a view to elicit information on sugar content due to increased N application and its influence on disease reaction in two paddy varieites, the present investigation was carried out.

Materials and Methods

Bhavani, moderately resistant to sheath rot and Kannagi, highly susceptible were raised in the field with three levels of nitrogen, *viz.*, 0, 65 and 100 N kg/ha. The plants were inoculated with the spore suspension (*ca.* 10^7 spores/ml), when they were 80 days old. Suitable controls were also maintained.

Leaf sheath samples were collected for analysis from healthy and inoculated plants, 1, 8 and 15 days after inoculation in the morning. The sheaths were extracted in boiling ethanol (Chandramohan *et al.*[3]).

Reducing sugar and the total sugar of the extract were estimated by Nelson's method[9]. The alcohol-extract was hydrolyzed with 1N H_2SO_4 (Inman[6]) for 30 min. at 49°C and after neutralizing the total soluble sugar content of the sample was obtained

Results

At higher levels of nitrogen, reducing sugars increased in both Kannagi and Bhavani. The quantity of reducing sugar, in general, was slightly higher in Kannagi than in Bhavani. Inoculation caused a slight reduction in reducing sugar level (Table I) The level of non-reducing sugars increased with increased nitrogen application. The quantity of non-reducing sugars was higher in Kannagi than in Bhavani. Inoculation slightly decreased the non-reducing sugar in both the varieties (Table II).

TABLE I

Changes in reducing sugars of rice cultivars due to* Acrocylindrium oryzae *inoculation and nitrogen fertilisation*
(mg/g dry weight)

Days after inoculation	Variety	N levels (kg/ha) 0.N H	0.N I	65.N H	65.N I	100.N H	100.N I
1	Kannagi	3·39	3·42 (+0·88)	3·62	3·57 (−1·38)	3·88	4·02 (+3·60)
	Bhavani	3·27	3·27 (0·00)	3·57	3·53 (−1·12)	4·27	4·30 (+0·70)
8	Kannagi	4·46	4·37 (−2·10)	4·74	4·26 (−10·13)	5·06	5·27 (+4·15)
	Bhavani	4·37	4·26 (−2·26)	4·40	3·91 (−11·14)	4·98	4·82 (−3·22)
15	Kannagi	4·81	4·42 (−8·11)	5·13	4·50 (−12·28)	6·46	6·02 (−6·82)
	Bhavani	4·96	4·87 (−1·83)	5·05	4·67 (−7·53)	6·42	5·76 (−10·50)

* Expressed in glucose equivalents.
Figures within the parentheses represent the per cent increase (+) or decrease (−) over healthy.

TABLE II

Changes in non-reducing sugars of rice cultivars due to* Acrocylindrium oryzae *inoculation and nitrogen fertilisation*
(mg/g dry weight)

Days after inoculation	Variety	N levels (kg/ha) 0.N H	0.N I	65.N H	65.N I	100.N H	100.N I
1	Kannagi	1·53	1·50 (−1·96)	1·84	1·76 (−4·35)	2;34	2·29 (−2·14)
	Bhavani	1·10	1·01 (−8·19)	1·25	1·36 (+8·80)	2·40	2·41 (+0·42)
8	Kannagi	2·36	2·10 (−11·02)	2·49	2·60 (+4·42)	3·91	3·65 (−6·65)
	Bhavani	2·15	1·80 (−16·28)	1·83	1·76 (−3·82)	2·74	3·00 (+6·00)
15	Kannagi	2·07	2·00 (−3·39)	2·88	2·74 (−4·86)	3·40	3·45 (+1·47)
	Bhavani	1·98	1·50 (−24·24)	2·92	2·42 (−17·13)	3·26	3·01 (−7·61)

* Expressed in glucose equivalents.
Figures within the parentheses represent the per cent increase (+) or decrease (−) over healthy.

Discussion

Generally, the sugar levels in the healthy and inoculated plants are correlated with resistance (Horsfall land Dimond[5]). In the present study, the levels of both reducing and non-reducing sugars increased in both Kannagi and Bhavani due to increased N application. The quantity of reducing and non-reducing sugars was higher in Kannagi than in Bhavani. High amounts of reducing and non-reducing sugars have been recorded in many plants susceptible to several pathogens (Otani[11], Ohata *et al.*[10]; Jayapal and Mahadevan[7]). Inoculation with the pathogen showed a reduction in reducing and non-reducing sugar levels in both Kannagi and Bhavani. Ranga Reddy and Sridhar[13] reported a same trend in rice plants infected with *Xanthomonas oryzae*.

The presence of more sugars in the tissue tends to increase susceptibility to invading pathogens as they serve sources of energy to the pathogen. So *A. oryzae* may be considered to be a "high sugar" disease. In several host parasite interactions, the levels of tissue sugars decrease following infection (Asada[1], Dayal and Joshi[4]). The reduction of sugars in the infected sheaths might be due to the utilization of these compounds by the pathogen and to the decreased synthetic ability of the severely infected leaves (Ranga Reddy and Sridhar[13]).

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ON THE ATYPICAL TRACHEARY ELEMENTS OF *ACACIA LEUCOPHLOEA* GALLS

THOUGH normality in form and structure is known to be a consequence of regular expression of morphogenetic phenomena controlled by genic or cytoplasmic factors (with an environmental impetus as well) involving a systematic transformation of meristematic cells into specialised cells of the plant body, occurrence of anomalous cell types especially in abnormal growth conditions such as those of galls induced by various organisms[1] and aseptic culture of tissue[2] appears to be of interest. Küster[1] and Mani[3] have elaborately reviewed the morphology and the possible functions of such anomalous cell types occurring in the instances of galls. Present investigation includes a comparative study of the tracheary elements of normal and galled shoot regions of *Acacia leucophloea* Willd. (Leguminosae).

Temporary preparations of serial transverse sections and macerations (by Jeffrey's method[4]) of the normal secondary tissues and woody galls of *A. leucophloea* were obtained, and were stained with aqueous toluidine blue. Diffuse porous normal wood of *Acacia* (Fig. 1) exhibited vessel elements of varied dimensions (120–150 × 40–50 μ) with dense alternate pitting, inclined or horizontal end plates (Fig. 3A) and occasionally a tail could be observed (Fig. 3C). The amorphous galls induced by *Haplophragmium ponderosum* Syd., on *A. leucophloea*[5] in transections revealed radiating, horizontally differentiating vascular system (Fig. 2), composed of shorter and thinner elements (80–90 × 20–30 μ) with annular or reticulate wall thickening (Fig. 3B) with the perforation generally occurring in one or both the terminals along the tangential wall (see also Refs. 6, 7) in addition to various other abnormal cell types. Anomalous tracheary cells have beeno bserved in the early stages of gall development, and they, with the ageing of the gall constitute an anastomosing network. Further, along the peripheral region of the galls, characteristic concentric rings of parenchyma cells were evident, which were gradually transformed into tracheary elements of earlier stated morphology, constituting vascular nests as observed in bacterial galls[8].

Distinctness in the morphology of the atypical xylem elements in galls compared with the normal ones of *A. leucophloea* appears significant. Although Wardlaw[9] attributes the induction of the new tissue to the diffusion of an 'exogenous substance', the reorientation