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SEED GERMINATION STUDIES WITH *CENCHRUS CILIARIS* L. II. ISOLATION AND CHARACTERISATION OF GERMINATION INHIBITORS FROM THE SPIKELETS

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IN *Cenchrus ciliaris* L, a prominent fodder grass of Indian arid zone, seeds (caryopsis) are enclosed within glumes in spikelets (dispersal unit) and the removal of caryopsis from the enclosing glumes has an enhanced effect on the per cent germination as compared to spikelets[1-4]. Inhibition of seed germination of the grass has been recorded by glume extract[2,3] and these inhibitors were believed to be coumarins[2]. Inhibition of seed germination by spikelet leachate was also recorded[4] and it seems that these inhibitors play an important role in restoring seed viability for a longer period. It was, therefore, decided to undertake the identification of the inhibitors associated with the dispersal unit of this range grass.

Spikelet leachate (obtained after soaking of spikelets in distilled water for 24 hr) examined for phenolics revealed the phenolic onium-ions (Anthocyanins[5]). Therefore, the spikelets were first extracted with petroleum ether to remove non-polar and non-phenolic substances and thereafter, the pigment was extracted in methanol-HCl (99:1 v/v) and purified chromatographycally[6]. A part of the purified pigment was acid-hydrolysed and aglycone (anthocyanidin) was collected in amyl alcohol and purified. The aqueous layer of the hydrolasate contains HCl, besides the sugar residue and before analysis the acid was removed from the sugar sample[8]. For determining the acyl residue of the pigment, spikelet leachate was hydrolysed with 2 NHCl for 5 hr at 100°C and filtered through Whatman No. 1 filter paper. The filtrate was then extracted[9] for phenolic substances soluble in ether and ethyl acetate.

The R_f values of the anthocyanin pigment, its aglycone and acyl residue were studied by descending chromatography on Whatman No. 1 paper. The solvents used for anthocyanin pigment were: BAW, *n*-butanol-acetic acid-water (4:1:5); Bu–HCl, *n*-butanol-2NHCl (1:1) and 1% HCl. The solvents used for the aglycone were: Forstal-acetic acid-conc. HCl-water (30:3:10); Formic-Formaic acid-conc. HCl-water (5:2:3). The solvents used for acyl residue (caffeic acid) were: BAW, butanol-acetic acid-water (63:10:27) and IBW, isopropanol-butanol-water (140:20:60). The sugar fraction of the pigment was also identified chromatographically by studying the anilin oxalate complex[10] of the sugar[11].

The results of the present work revealed that the R_f and λ_{max} values of the extracted pigment and its aglycone tallied closely with those of 3-monosidic glycoside of cyanidin and authentic cyanidin. The R_f ($\times 100$) values for the pigment were 42 (BAW), 32 (Bu HCl) and 05 (1% HCl) and absorption maxima in Me-OH-HCl were 274 (UV) and 523 (visible) respectively. The values for the aglycone are 49 (Forestal) and 22 (Formic) and λ_{max} values 277 (UV) and 535 (visible) respectively. The R_f ($\times 100$) value of the sugar is 33 (ethyl acetate-pyridine-water, 2:1:2) and the colour of its aniline oxalate complex[11] was similar to those of standard arabinose. Finally, the R_f ($\times 100$) value of its acyl residue (caffeic acid) are 81 (BAW) and 72 (IBW), which gives brown colour with diazotesed *p*-nitroanaline. The pigment fluoresces in UV light and gives dull magenta colour which may be considered as an additional proof for the pigment being a 3-glycoside-a monoside. Thus the inhibitor (pigment) has the following structural features. (1) The aglycone part of the pigment is cyanidin. (2) The pigment is a 3-glycoside-a monoside. (3) The sugar present in the pigment is arabinose. (4) Acylated residue is caffeic acid. Therefore, the pigment is cyanidin-3-arabinoside, acylated with caffeic acid.

Phenolics and coumarins are often reported as almost universally present inhibitors, which also act as germination inhibitors in seed husks, coats, fruits etc[12]. Flavonoids with a basic C_6-C_3-C_6 skeleton are widespread in seed plants and both aglycones and glycosides are extremely potent toxins to seed germi-

nation[14] and much evidence has accumulated indicating that phenolic compounds of several type are important in the resistance of plants to infection by fungal, bacterial and viral diseases[13,14]. *Thus, there is a clear evidence that these germination inhibitors associated with seed endosing glumes are compounds that are microbial inhibitors also, which seems to play an important role in prevention of seed decay before germination in natural semi-arid areas as most seeds that do not germinate rapidly after landing in soil would be decomposed before germination*, especially in the case of long unfavourable environmental condition, if they did not contain these microbial inhibitors—phytoncides. Therefore, this may be one of the most consistent and important ecological role of allelopathy in natural ecosystems, although the limited amount of research does not reflect its importance[14] and it appears that this is a fruitful phase of allelopathy for future research[15].

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RELATIONSHIP BETWEEN CHROMOCENTRES AND CHROMOSOMES IN CRUCIFERAE

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MEMBERS of the family Cruciferae are characterized by the presence of chromocentres in the interphase nuclei[1–3]. They are seen as dark staining heteropycnotic bodies and represent pericentric constitutive heterochromatin. Over the past decade Dayal[3–6] has made extensive cytogenetical studies on chromocentres in the cultivated radish, *Raphanus sativus* L. Localized heterochromatic chromocentres have been considered an adaptive character and attributed considerable evolutionary value[1,7]. The mean chromocentre frequency is known to be characteristic for a species or even a population. However, there is some evidence that it varies and depends upon chromosome length, genome size, degree of polyploidy and genetic factors[3–6,8]. Crucifers may be modelled for such studies. The present study has, therefore, been undertaken to see whether chromocentre frequency bears any relationship with chromosome number in the members of Cruciferae.

Seven species belonging to four genera of the family Cruciferae constituted the material for the present study (table 1). Chromosome number of these species

Table 1 *Chromocentres per nucleus and chromosome number (2n) in Cruciferae*

Materials	Chromosome number (2*n*)	Chromocentres per nucleus
Iberis amara L.	14	14.1
Brassica nigra Koch.	16	11.8
Raphanus sativus L.	18	13.6
Brassica campestris L.	20	14.3
Eruca sativa L.	22	16.2
Brassica alba Boiss.	24	15.4