11. Carcellar, M. and Fraschina, A., *Bot. Gaz.*, 1986, **147**, 98.
12. Greenway, H. and Munns, R., *Annu. Rev. Plant Physiol.*, 1980, **31**, 149.
13. Schobert, B. and Techesche, H., *Biochem. Biophys. Acta.*, 1978, **541**, 270.
14. Lee, Y. P. and Takahashi, T., *Anal. Biochem.*, 1966, **14**, 71.
15. Consden, R., Gordon, A. H. and Martin, A. J. P., *Biochem. J.*, 1944, **38**, 225.
16. Tyankova, L. A., *C. R. Acad. Bulg. Sci.*, 1966, **19**, 847.
17. Hubac, C., *C. R. Acad. Sci. (Paris)*, 1967, **264**, 1286.
18. Goas, M., *C. R. Acad. Sci. (Paris)*, 1965, **261**, 2724.

GENETICS OF PHENOL COLOUR REACTION OF SEED COAT IN PEARL MILLET [*PENNISETUM AMERICANUM* (L.) LEEKE]

Y. SAIDESWARA RAO*, V. MANGA and M. V. SUBBA RAO
Botany Department, Andhra University, Waltair 530 003, India.
**Present address: Genetic Resources Unit, ICRISAT, Patancheru 502 324, India.*

PHENOTYPIC markers, which have no apparent selective advantage, can serve as useful criteria in understanding the geographical and evolutionary paths of divergence that a system has undergone before it attained its present status. Colour change of the seed coat in response to treatments with phenol is one such character. This phenol colour reaction of seeds was first reported in wheats[1] and since then has been in use for testing the purity of a variety in seed testing procedures[2]. The test was reported to be indicative of the genotype and independent of the growth conditions, provided the grain ripens to $<30\%$ of moisture content[3].

Despite its suggested usefulness in understanding the phylogenetic differentiation and geographical distribution of plant systems, studies on the genetic basis of this character are limited to rice[4, 5], wheat[6, 7], rye[3], and foxtail millet[8] and no information seems to be available about the phenol colour reaction in pearl millet (*Pennisetum americanum* (L.) Leeke). In the present study eight inbreds of pearl millet are classified basing on the reaction of their seed coats to phenol colour test and the genetic basis of this character is analysed.

Dry and dehusked grains of pearl millet [*P. americanum* (L.) Leeke] (= *P. typhoides* Stapf. and Hubb.; $2n=14$) were placed on filter paper in a petri dish. The filter paper was moistened with 3 ml of 1% aqueous phenol solution. The petri dish was kept at 30 ± 2°C and was covered to prevent loss of moisture and left for 3 h for completion of the colour reaction. The grains were then gently dried by keeping on blotting paper and the colour of the treated grains due to the reaction—whether normal pearly or dark brown—was noted.

Since the site of colour reaction is a maternal tissue, the F_1 grains used for these studies were those borne by the F_1 plants and F_2 grains borne by F_2 plants. For example the F_2 population was formed by taking grains from each of the F_2 plants.

Concentration of the phenol solution and duration of the treatment were standardized after several trial experiments. With the particular staining schedule used in the present study, no loss in seed viability was observed and the percentage germination of the treated seed was comparable to that of the control (untreated). In plants raised from the treated seed, no chromosomal aberrations were observed either at root tip mitosis or at the PMC meiosis. The progeny of the treated seed was studied through four generations of selfing and no gene mutations were recorded.

Eight inbreds (Vg 212, Vg 587, IP 1746, IP 1475, IP 3128, LC.I, LC.II and CO_5) were examined for their reaction to phenol colour test and only one inbred (IP 3128) showed negative phenotype, in that the colour of the seed coat remained unchanged even after the test. The remaining seven inbreds showed positive reaction to the test and the colour of the seed coat turned dark brown after treatment. Both positive and negative phenotypes observed in different inbreds were quite stable since the character was observed through several generations of selfing. No seasonal differences in the expression of this character were observed. In all seven inbreds exhibiting positive phenotype, the intensity of dark brown colour developed, appeared to be identical.

To study the genetic basis of this character, reciprocal crosses were made between plants showing negative and positive phenotypes and the data are presented in table 1. The phenotype observed in F_1 was always positive. In the F_2 generation, segregation was observed for the positive and negative

Table 1 *Data on the inheritance pattern of phenol colour reaction of the pericarp in pearl millet*

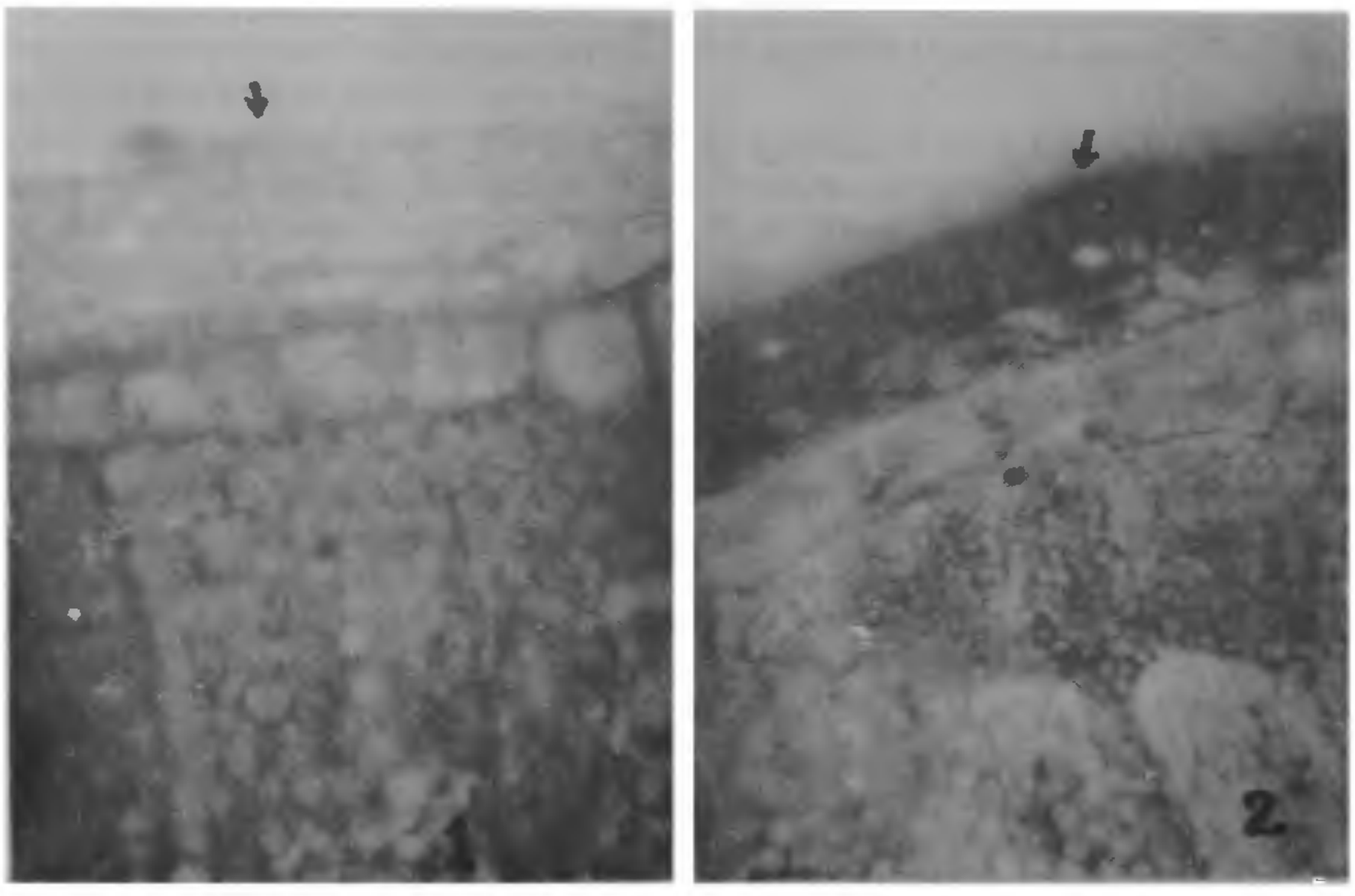
Cross	Phenotype of F_1	Segregating lines of phenotypes in F_2		χ^2 (3:1)
		+ve	−ve	
IP 3128 ♀ × Vg 587 ♂	+ve	78	34	1.71
Vg 587 ♀ × IP 3128 ♂	+ve	80	23	0.3916
IP 3128 ♀ × CO_5 ♂	+ve	45	18	0.4285
CO_5 ♀ × IP 3128 ♂	+ve	63	20	0.0361

phenotypes which was a good fit to 3:1 ratio ($P<0.05$). No differences in reciprocal crosses were observed.

The reaction of the seed coat of eight accessions of pearl millet to phenol colour test was a stable genetic character since the same phenotype or reaction whether positive (+ve) or negative (−ve) was observed in four successive generations. Phenol colour reaction was found to depend on the moisture content of the seed[3]. Though no quantitative estimation of the seed moisture content was made during the present study, the seeds harvested in different seasons showed consistency in the expression of this colour reaction indicating that with the conventional practices of harvesting and seed drying, this character can serve as a useful genetic marker.

The concentration of phenol and the duration of treatment used in the present study did not cause any chromosomal or genetic changes as evidenced from the absence of macro- or micro-mutations in the plants raised from the treated seed or in their subsequent selfed progeny. However, in terms of viability factors, phenol treatment was deleterious when seeds were exposed to higher concentrations or over longer periods in low concentrations. Lower concentrations and shorter periods of treatment resulted in low intensity of colour and non-uniform staining of the seed coat which sometimes lead to ambiguity in the assessment of colour reaction.

The pericarp in all these inbred lines was normally colourless. The original pearly colour of the grains, as is known in maize[9], is a quality derived from the



Figures 1 and 2. Seed coat reaction to phenol test. **1.** Section showing colourless pericarp in untreated grains and grains of negative phenotype, and **2.** Section showing coloured pericarp in treated grains of positive phenotype.

endosperm[10]. In the present study when the grains were exposed to phenol solution, only the pericarp showed colour development (dark brown) while the endosperm showed no colour change (figures 1 and 2). Thus the phenol colour reaction was confined only to the pericarp (pre-fertilization tissue).

From the results presented in table 1, it is evident that phenol colour reaction is controlled by a single gene. Similar genetic studies have been carried out on phenol colour reaction in other crop plants and these indicated that this character was controlled either by a single gene with two allelomorphs as in rice[5] and *Setaria italica*[8] or by a single locus with multiple alleles as in Emmer wheats[7] or by one or two dominant genes as in bread wheat[11]. In pearl millet, the positive phenotype is the dominant one and the gene symbol *Phc* is proposed.

In view of its genetic stability, the phenol colour reaction of the seed coat in pearl millet can be a useful marker to trace the geographical distribution of various land races and in seed identification procedures.

The authors thank Dr M. H. Mengesha and Dr S. Appa Rao, Genetic Resources Unit, ICRISAT, Patancheru for the supply of IP lines and Prof. B. G. S. Rao, Department of Botany, Andhra University, for helpful discussions. Financial assistance from UGC, New Delhi, is gratefully acknowledged.

27 April 1988

1. Pieper, H., *Dtsch. Landwirtsch. Presse.* 1922, **67**, 438.
2. ISTA Rules, *Proc. Int. Seed Test. Assoc.*, 1966, p. 31.
3. Wrigley, C. W. and Mc Intosh, R. A., *Wheat Inf. Serv.*, 1975, **40**, 6.
4. Oka, H., *Jpn. J. Breed.*, 1953, **3**, 33.
5. Morinaga, T., Nagamater, T. and Kawahara, E., *Jpn. J. Genet.*, 1943, **19**, 206.
6. Bhowal, J. G., Benerjee, S. K. and Joshi, M. G., *Jpn. J. Genet.*, 1969, **44**, 123.
7. Joshi, M. C. and Benerjee, S. K., *Indian J. Genet.*, 1969, **29**, 280.
8. Kawase, M. and Sakamoto, S., *Theor. Appl. Genet.*, 1982, **63**, 117.
9. Kiesselbach, T. A., *Res. Bull. 161 Agric. Exp. Station*, Nebrasko College of Agriculture, 1949, Reprinted 1980, Nebraska Press.
10. Phul, P. S., Athwal, B. S. and Gill, B. S., *Indian J. Genet.*, 1969, **29**, 438.
11. Miczynski, K., *Z. Pflanzenzucht.*, 1938, **22**, 564.

POLYSTICHUM MAKINOI (TAG.) TAG.—A NEW RECORD FOR INDIA

N. PUNETHA and B. S. KHOLIA
Department of Botany, Government P.G. College, Pithoragarh 262 501, India.

THE dryopteroid genus *Polystichum* is fairly widespread in the Indo-Himalayas. In the Western Himalayas itself, the genus is represented by 23 species[1-3] of which 13 are found in Pithoragarh district, the easternmost section of the Western Himalayas. During our survey we collected one more species, *P. makinoi* (Tag.) Tag., from Dhaj, Munsyari and Namik (2400–2800 m). A perusal of the recent literature on the taxonomy and distribution of the species[1-3] shows that this species has not been reported from India although it was known from Nepal and Bhutan[4]. This species is widely distributed in S.E. China, Japan and Tibet[4,5].

P. makinoi is similar to two other Indian species *P. discretum* (D. Don) J. Smith and *P. piceo-paleaceum* Tag. *P. makinoi* differs from the former in having broad dark-coloured scales on stipe and rachis, and coriaceous lamina, concolorous and slightly dentate scales on stipe and rachis differentiate *P. makinoi* from *P. piceo-paleaceum* where the scales are restricted to stipe base and are bicolorous with ciliate margins.

Specimens examined: Punetha Pith. 1201, 1202, 1203, 1630; CAL Date of collection: July 1986, August 1987.

The authors thank Dr C. R. Fraser-Jenkins (England) for determining the species and for suggestions. Financial help from UGC, New Delhi, is gratefully acknowledged.

30 April 1988; Revised 2 September 1988

1. Khullar, S. P., *J. Indian Bot. Soc.*, 1986, **65**, 38. (Abstract).
2. Khullar, S. P., *Proc. Indian Sci. Congr. Assoc.*, 74th session, 1987, p. 95, (Abstract).
3. Khullar, S. P., *Indian Fern J.*, 1987, **4**, 28.