Theor. Appl. Genet. 63, 117–119 (1982)

Geographical Distribution and Genetic Analysis of Phenol Color Reaction in Foxtail Millet, *Setaria italica* (L.) P. Beauv. *

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Summary. Phenol color reaction was examined in a total of 376 strains of foxtail millet, *Setaria italica* (L.) P. Beauv., collected from different areas throughout Eurasia. Positive and negative phenotypes, and no intermediate type could be recognized by the phenol color reaction. Of 376 strains examined, 50 were positive, 319 were negative, five were mixtures of both phenotypes, and the coloration in two strains with blackish lemmata and paleae could not be distinguished. The strains that showed the positive phenotype of phenol color reaction were found in rather limited regions, while those with the negative phenotype occurred in almost all the regions. The positive phenotype occurred more frequently in the lower latitudinal regions of Asia. Genetic analysis of the F_1 and F_2 generations between the two phenotypes showed that the phenol color reaction is controlled by a single gene, and that the positive phenotype is dominant.

Key words: Phenol color reaction – Geographical distribution – *Setaria italica*

Introduction

The phenol color reaction, which was first reported by Pieper (1922) in wheats and others, is a coloration occurring in caryopsis organs produced with a phenol solution. In Asian cultivated rice, the phenol color reaction was used by Oka (1953) as one of the key characters for distinguishing between the two variety groups, the continental type and the insular type.

Since no color reaction is produced when the organs are not in contact with phenol, the coloration would not serve as a direct means of selection by humans for agricultural practices. Although it is not still clear whether any interrelationships exist between the phenol color reaction and adaptational process, the phenol color reaction might be helpful in the studies on phylogenetic differentiation and the geographical distribution within the intraspecific variation.

Foxtail millet, *Setaria italica* (L.) P. Beauv., is an old staple crop in Eurasia, and is thought to have been one of the main cultivated cereals in the early period of agriculture. It is now cultivated sporadically on a small scale in scattered local areas throughout Eurasia as a relict crop. A given strain of *S. italica* collected from a local area can be generally regarded as an endemic race that has been grown in that area for a long time. Thus, *S. italica* is considered to be a desirable material to study the phylogenetic differentiation of crops and related problems.

The present study was made using *S. italica* to investigate the geographical distribution of the phenol color reaction and to determine the genetic basis of this character.

Materials and Methods

In the present study, 376 strains of foxtail millet, *Setaria italica* (L.) P. Beauv., were used. They were originally collected from Japan, Korea, China, Taiwan, the Philippines, Thailand, Indonesia, Nepal, India, Afghanistan, the USSR and Europe, as listed in Table 1. Of the 376 strains, 290 were collected directly from the local villages where they had been cultivated. The remaining 45 strains from China, 30 from India, seven from the USSR, and four from Europe were provided by the various institutes as described in Table 1. All strains were maintained at the Plant Germ-plasm Institute, Kyoto University.

Ten mature grains with lemmata and paleae for each strain were soaked in 2 ml of a 3% phenol solution for 24 hours at room temperature. Then, they were gently dried. Whether or not the grain surfaces were stained blackish brown was recorded.

To analyze the genetic basis of phenol color reactions in *S. italica,* two strains with different phenotypes, positive and negative, from Taiwan were crossed by the method of Takahashi (1942). The phenol color reaction of the F_1 and F_2 progenies was analyzed by the procedure previously outlined.

* Contribution No. 28 from the Plant Germ-plasm Institute, Faculty of Agriculture, Kyoto University, Kyoto (Japan)

0040-5752/82/0063/0117/$ 1.00

Table 1. Strains used

Region	No. of strains used	Collection locality or source (No. of strains)
Japan		
Honshu Is.	42	Iwate Pref. (2); Niigata Pref. (1); Nagano Pref. (5); Yamanashi Pref. (6); Gifu Pref. (2); Kanagawa Pref (1); Mie Pref. (1); Nara Pref. (23); Hyogo Pref. (1).
Shikoku Is.	33	Kochi Pref. (24); Tokushima Pref. (5); Ehime Pref. (4).
Kyushu Is.	18	Kumamoto Pref. (12); Ōita Pref. (1); Miyazaki Pref. (5).
Nansei Isls.	28	Tanegashima Is. (1); Okinawa Is. (2); Ogami Is. (2); Tarama Is. (1); Irabu Is (2); Ikema Is. (4); Ishigaki Is. (5); Taketomi Is. (4); Iriomote Is. (4); Hateruma Is. (2); Yonaguni Is. (1).
Korea	96	Kangwon-do (5); Kyonggi-do (12); Chungchong-pukto (8); Chungchong-namdo (5); Kyongsang-pukto (9); Kyongsang-namdo (8); Cholla-pukto (8); Cholla-namdo (21); Cheju-do (10).
China	45	Tohoku Nat. Agr. Exp. Sta., Japan (20); Nat. Inst. Agr. Sci., Japan (25).
Taiwan	39	Pingtung (26); Hwalien (1); Lan Hsu Isls. (12).
Philippines	13	Itbayat Is. (5); Batan Is. (7); Luzon Is. (1).
Indonesia	5	Halmahera Is. (5).
Thailand	1	A village near Cheng-mai (1).
Nepal	3	Dhunche (1); Bhargu (2).
India	30	Univ. Agr. Sci., Bangalore, India (20); T.N.A.U., Coinbatore, India (10).
Afghanistan	12	Jabalsalaj-Zenya (2); Kabul (4); Takhar (2); Badakhshan (4).
USSR	7	N. I. Vavilov All-Union Inst. Plant Industry, Leningrad, USSR [Dagestan (1); Kirghizia (2); Uzbekistan (1); Primorskaya Prov. (1); Georgia (1); Ukraine (1)].
Europe	4	Dienst voor Parken en Plantsœnen, Antwerpen, Belgium (3); Inst. Genet. Plant Breed., Prague-Ruzyne, Czechoslovakia (1).
Total	376	

Results and Discussion

Variation and Geographical Distribution of Phenol Color Reaction

Two phenotypes were recognized as a positive and a negative phenol color reaction in mature grains of *S. italica* used in the present study, the positive reaction being blackish brown and the negative reaction showing no sign of coloration. There was little variation in color intensity within each phenotype; no intermediate type was found. It is in marked contrast to the broad variation ranging from none or slight to deep coloration reported by Kondo and Kasahara (1940) and others for the phenol color reaction of wheats and barley.

Of the 376 strains examined in this study, 50 were positive, 319 were negative, five were mixtures of both phenotypes; the remaining two could not be identified, as summarized in Table 2. The strains with the positive color reaction were found in rather limited regions, while those with the negative one occurred in almost all the regions so far examined. The positive phenotype was not found in the strains from China. It was seldom found in Japanese or Korean strains, while it was found in almost half of the strains from Taiwan and the Philippines, and about one third of Indian strains. There is an interesting tendency that the positive phenotype occurred more frequently in lower latitudinal regions of Asia. The phenol color reaction of two strains collected from the Lan Hsu Islands of Taiwan could not be determined because of the blackish color of lemma and palea.

There might be three distributional gaps for the positive phenol color reaction: one between Japan and Taiwan, another between China and Taiwan, and the other between China and India. On the other hand, the strains with the negative phenotype were widely distributed throughout Eurasia. In *Oryza sativa,* the strains with the positive phenotype have been more frequently found in South China, Burma, Thailand, Nepal and India, while they have been seldom found in Japan, North China or Indonesia (Iizuka et al. 1977). It is quite interesting to point out that the distribution patterns between *S. italica* and *O. sativa* are similar in frequencies of the strains with the positive phenotype. These two cereal crops might be similar therefore in certain ways such as in their dispersal patterns or in their manner of reaction to particular environmental factors.

Since the phenol color reaction would not serve as a direct means of human selection for *S. italica,* which can be regarded as a relict crop, the geographical distribution of this character might indicate the dispersal pathways of this millet in adjacent areas. As shown in Table 2 only a few Japanese strains with the positive phenotype were collected, all from four small adjacent islands, Ikema, Irabu, Tarama, and Ishigaki, in the southern extremity of the Nansei Islands. It might be assumed, therefore, that an introduction of strains with the positive phenotype took place from Taiwan, located about 200 km west of these islands, where a high frequency of the strains with the positive phenotype was observed.

Table 2. Summary of phenol color reaction in *S. italica*

Region	No. of strains used	Positive		Negative		Mixture		Not determined	
		No.	(%)	No.	(%)	No.	(%)	No.	(%)
Japan									
Honshu Is.	42	–	–	42	(100.0)	–	–	–	–
Shikoku Is.	33	–	–	33	(100.0)	–	–	–	–
Kyushu Is.	18	–	–	18	(100.0)	–	–	–	–
Nansei Isls.	28	6	(21.4)	22	(78.6)	–	–	–	–
Subtotal	121	6	(5.0)	115	(95.0)	–	–	–	–
Korea	96	2	(2.1)	94	(97.9)	–	–	–	–
China	45	–	–	45	(100.0)	–	–	–	–
Taiwan									
Taiwan Is.	27	17	(63.0)	10	(27.0)	–	–	–	–
Lan Hsu Isls.	12	6	(50.0)	4	(33.3)	–	–	2	(16.7)
Subtotal	39	23	(59.0)	14	(35.9)	–	–	2	(5.1)
Philippines	13	6	(46.2)	7	(53.8)	–	–	–	–
Indonesia	5	–	–	5	(100)	–		–	–
Thailand	1	–	–	1	(100)	–	–	–	–
Nepal	3	3	(100)	–	–	–	–	–	–
India	30	9	(30.0)	19	(63.3)	2	(6.7)	–	–
Afghanistan	12	–	–	10	(83.3)	2	(16.7)	–	–
USSR	7	–	–	7	(100.0)	–	–	–	–
Europe[a]	4	1	(25)	2	(50)	1	(25)	–	–
Total	376	50	(13.3)	319	(84.8)	5	(1.3	2	(0.5)

[a] Belgium and Czechoslovakia

Genetic Analysis of Phenol Color Reaction

The F_1 hybrid between the two phenotypes showed a positive phenol color reaction, which indicates that the positive color reaction is dominant. The F_2 generation showed a segregation of 297 positive and 91 negative, which can be taken to show no significant deviation from an expected phenotypic ratio of 3 positive : 1 negative in F_2 ($x^2=0.495$; $P=0.5–0.3$). The result indicates that this character is controlled by a single gene, with the positive phenotype being dominant, as reported in rice by Morinaga et al. (1943). On the contrary, this character in wheats seems to be more complicated. Two completely linked dominant genes, one controlling the positive phenol color reaction in spikes and the other in kernels were suggested to exist in wheats by Fraser and Gfeller (1936). Miczyński (1938) revealed that one or two dominant genes control the positive phenol color reaction in kernels and another single dominant gene in spikes of wheats.

Acknowledgement

The authors express their sincere thanks to all those who have kindly provided seed samples for the study.

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Received May 1, 1982
Accepted July 2, 1982
Communicated by D. von Wettstein

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