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Observations on the biology of flowering of wild forms from the subgenus *Glycine*

HALINA SKORUPSKA, JERZY NAWRACAŁA

Chair of Plant Genetics and Breeding, Academy of Agriculture, Wojska Polskiego 71c, 60-625 Poznań, Poland

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Abstract

Wild forms from the subgenus *Glycine* were collected and cultivated from 1977. In total, 45 forms were gathered. Observations on the degree of variability of traits connected with the biology and effectiveness of flowering were made. Studies were done on the process of pollination and pollen tube growth *in vivo* in cleistogamic and chasmogamic flowers. As a result of these analyses, it was found that the phenomenon of very poor pod setting in racemes may be connected with disturbances in the process of pollination.

Key words: Glycine, cleistogamic flowers, chasmogamic flowers, biology of flowering

INTRODUCTION

The genus *Glycine* is divided into two subgenera: *Soja* and *Glycine*. The domesticated soybean *G. max* L. Merr. and *G. soja* Sieb. and Zucc. belong to subgenera *Soja*; the other encompasses six species: *G. tabacina* (L.) Benth., *G. tomentella* Hayata, *G. canescens* F. J. Herm., *G. clandestina* Wendl., *G. falcata* Benth., *G. latrobeana* Meissn. Benth. (Hymowitz and Newell 1981). The domesticated soybean, *Glycine max*, is characterized by a high degree of variability which enables it to adapt to both tropical and temperate climates. *Glycine soja* forms are found growing naturally in The Peoples Republic of China, Taiwan, Japan and the Asian regions of the USSR. Wild forms of *Glycine* are distributed mainly in Australia, but have also been found in Southern Peoples Republic of China, Taiwan, on South Pacific Islands, the Phillipines and New Guinea (Hymowitz 1970).

During recent years, interest has grown in the wild forms of species belonging to the genus *Glycine* because they constitute a source of genes of an exceptionally high selective value: resistance to pathogenes, resitance to unfavorable environmental conditions, drought, high soil salt-content, the high protein content and the protein and oil composition of its seeds. The possibilities of increasing hereditary variability through interspecific crosses within the subgenus *Soja* (Hymowitz et al. 1972, Kaizuma 1975, Fukui and Sanaga 1978, Kaizuma and Fukui 1978, Taira et al. 1978, Broich and Palmer 1980) have become relatively well determined. Interesting wild and semi-wild soybean forms from the subgenus *Soja*, suitable for crosses under Polish climatic conditions, have also been selected (Skorupska 1981). Few studies deal with the problem of interspecific crosses within subgenus *Glycine* or with the possibility of obtaining hybrids of these species with the domesticated soybean (Ladzinsky et al. 1979, Newell and Hymowitz 1982). This study is aimed at presenting observations on the biology of blooming of wild froms from the subgenus *Glycine* derived from different climatic regions. An attempt was also made to explain the lowered effectiveness of blooming of the racemes of these species.

MATERIAL AND METHODS

The plant material was collected and cultivated from 1977 to 1980. Seeds of wild species of the subgenus *Glycine* were obtained from the University of Illinois, Urbana, USA, the University of Morioka, Japan, the WIR, Leningrad, USSR, and from several Australian sources. In all, 45 froms were obtained (Table 1).

Table 1

Number of the subgenus *Glycine* species by origin

Species	Source				
	Australia	Japan	USA	USSR	total
G. canescens	1	1	2	2	6
G. clandestina	2	5	3	—	10
G. falcata	—	—	1	—	1
G. tabacina	2	12	2	2	18
G. tomentella	3	4	1	2	10
Total	8	22	9	6	45

Pot experiments were run in greenhouses in 1981 and 1982 (in triplicate, 10 plants per repetition). Before planting, the seeds were scarified in order to eliminate eventual differences in their germination. The observations

dealt with the vegetation of the plants, with special attention being paid to to the blooming phase. Basic observations of the flowers of the first five racemes were conducted. The flower buds were gathered at different stages of development: 1) about 1 week before blooming, 2) buds with closed corollas, 3) the beginning of the unfolding of petals, 4) full-bloom, 5) 1 day after the flower opened, 6) 2 days after the flower opened, 7) 3 days after opening, 8) slightly browned anthers. The observations were done on about 20 flowers in each stage.

The course of pollination and pollen tube growth *in vivo* was followed using a fluorescent microscope. The best microscopic images were obtained using the following method: flower buds were fixed in a mixture of C_2H_5OH:$CHCl_3$:CH_3COOH (6:3:1) for 24 h at a temperature of +4°C. They were stored in 70% ethyl alcohol. Next, pistils and stamens were isolated and macerated in 1 N NaOH for 2.5 h at 60°C. After maceration, the objects were carefully rinsed twice under running water and once in distilled water. They were then stained in 1% aniline blue for 24 h. Fluorescent microscopic observations were done in a drop of glycerine.

The effectiveness of blooming was defined as the ratio of the number of pods to the number of flowers in the analysed raceme.

RESULTS AND DISCUSSION

Seedlings were observed 3-5 days after the seeds had been sown at the beginning of the second decade of April. During growth, the plants formed rosettes, from which long, climbing stems differentiated. Only *Glycine falcata* plants had a stiff, erect shape. The plants began to bloom very early, during the third decade of May.

In most forms, after 45-62 days, the axillary flowers bloomed first. After 2-3 weeks, the racemes began to bloom (Table 2). From among all of the collected species, only one form, *Glycine tomentella* 1349, did not enter the stage of generative development. The value of the variance coefficients of the beginning of blooming of flowers in axils and blooming of racemes were very small for individual genotypes (Table 2). Jaranowski et al. (1980) estimated the variability range of several developmental characteristics in over 400 *Glycine max* forms and found that the dates of blooming were similar during the 4 years of observations. Strong genetic determination of the beginning of blooming seems to be a characteristic trait of the genus *Glycine*.

In most of the observed forms, a decided dimorphism of flowers was found (cleistogamic flowers and chasmogamic flowers in racemens). First, in the axils, single (less often, 2-3 grouped together), tiny flowers on short pedicels were developed (Fig. 1). Their perianth was highly

Table 2

Characteristics of flowering period of raceme-producing forms from the subgenus *Glycine* (day after sowing)

Form	Beginning of flowering of axillary flowers				Beginning of recemes flowering				Termination of recemes flowering			
	1981	1982	$\bar{x}$	V%	1981	1982	$\bar{x}$	V%	1981	1982	$\bar{x}$	V%
Glycine canescens												
401	48	51	49.5	3.0	63	62	62.5	0.8	90	88	89.0	1.1
Glycine clandestina												
436	48	51	49.5	3.0	61	68	64.5	5.4	90	91	90.5	0.5
449	48	52	50.0	4.0	72	67	69.5	3.6	91	86	89.0	0.5
Glycine falcata												
PI 233 139	0	0	0	0	60	62	61.0	1.6	85	90	87.5	2.8
Glycine tabacina												
Chinchilla	52	49	50.5	2.9	61	66	63.5	3.9	94	81	87.5	7.4
Giken	50	48	49.0	2.0	71	67	69.0	2.9	94	91	92.5	1.6
Helidon	50	48	49.0	2.0	71	66	68.5	3.6	94	91	92.5	1.6
Hutton	46	44	45.0	2.2	77	76	76.5	0.6	94	88	91.0	3.3
Miyakojima tsurumame	61	63	62.0	1.6	74	74	74.0	0	90	84	87.0	3.4
Miles	50	48	49.0	2.0	61	66	63.5	3.9	94	87	90.5	3.9
Taichung 4	51	52	51.5	0.9	66	65	65.5	0.8	94	81	87.5	7.4
1077	50	52	51.0	1.9	69	72	70.5	2.1	94	93	93.5	0.5
1258	54	52	53.0	1.9	73	75	74.0	1.3	94	90	92.0	2.2
PI 193232	51	53	52.0	1.9	66	68	67.0	1.5	94	91	92.5	1.6
PI 248253	49	52	50.5	2.9	71	69	70.0	1.4	94	88	91.0	3.3
PI 272099	58	55	56.5	2.6	66	68	67.0	1.5	94	90	92.0	2.2
PI 319697	51	52	51.5	0.9	71	65	68.0	4.4	91	92	91.5	0.5
PI 378707	50	52	51.0	1.9	61	67	64.0	4.7	94	89	91.5	2.7
WIR 377157	48	48	48.0	0	61	62	61.5	0.8	94	89	91.5	2.7
WIR 367178	48	48	48.0	0	61	68	64.5	5.5	94	89	91.5	2.7
Glycine tomentella												
Eskdale	60	58	59.0	1.7	91	85	88.0	3.4	105	96	100.5	4.5
Inverelle	54	58	56.0	3.6	68	70	69.0	1.4	87	94	91.0	3.3
486	60	62	61.0	1.6	85	90	87.5	2.8	101	105	103.0	1.9
PI 245332	46	50	48.0	4.2	60	57	58.5	2.6	105	98	101.5	3.4
PI 319696	51	55	53.0	3.8	63	69	66.0	4.5	85	88	86.5	1.7
WIR 367177	51	53	53.5	4.7	67	70	68.5	2.2	81	85	83.0	2.4

$\bar{x}$ — average values of traits, V — variance coefficient of analysed traits.

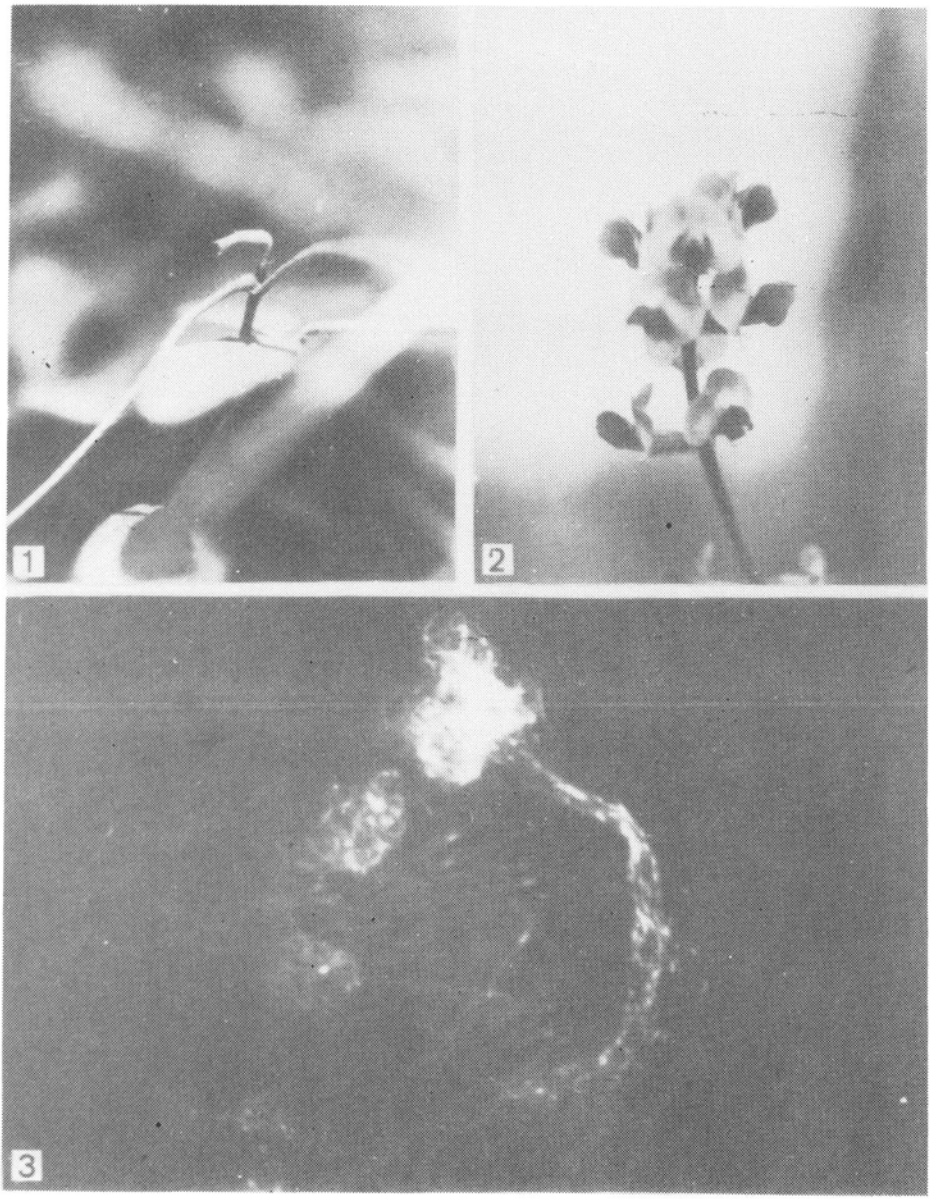

PLATE 1

Fig. 1. A single cleistogamic flower from *Glycine clandestina* PI 245252. x 1. Fig. 2. Chasmogamic flowers on raceme from *Glycine tomentella* PI 245332. x 1.5. Fig. 3. A bundle of pollen tubes in the pistil of a cleistogamic flower. x 32

reduced, the petals light-green, the pistil style strongly curved, which caused the stigma to almost touch the upper part of the ventral side of the ovary. During blooming, the stamens grew to the level of the stigma and tightly enveloped it. This extremely favored self-pollination, even more so, since unfolding of the reduced petals was never observed. Cleistogamic flowers such as these were found in all of the species except *Glycine falcata*. Most of the *G. clandestina* and *G. canescens* forms in our possesion produced only axillary flowers (Table 3). Also, in two late-blooming *G. tomentella* forms (90 and 104 days after sowing) only cleistogamic flowers were observed. In all, from the 45 collected forms, 42.2% formed only cleistogamic flowers. For technical reasons, their suitability for crossing

Table 3

Variation in the flowering of forms of subgenus *Glycine* characterised by producing only cleistogamic flowers

Form	Day from sowing to bloom of axillary flowers				Percentage of forms not producing racemes
	1981	1982	$\bar{x}$	V	
Glycine canescens					83.3
1240	45	48	46.5	3.2	
1339	48	45	46.5	3.2	
PI 399478	45	48	46.5	3.2	
WIR 367 125	45	46	45.5	1.1	
WIR 367190	46	48	47.0	2.1	
Glycine clandestina					80.0
Iwate University	45	47	46.0	2.2	
1001	45	47	46.0	2.2	
1074	45	51	48.0	6.2	
2743	46	44	45.0	2.2	
PI 233138	45	52	48.0	7.2	
PI 245252	48	47	47.5	1.0	
PI 245745	46	52	49.0	6.1	
PI 246590	48	48	48.0	0	
Glycine tabacina					11.1
Bokho tsurumare	52	53	52.5	0.9	
1336	61	58	59.5	2.5	
Glycine tomentella					40.0
1133	108	100	104.0	3.8	
1188	92	88	90.0	2.2	
1349	0	0	0	0	
WIR 367 176	60	62	61.0	1.6	

0 — did not flower, $\bar{x}$ — average values of traits, V — variance coefficient of analysed traits.

seems to be small. In 1979, in order to prevent the loss of the *Glycine tomentella* Hayata PI 393567 genotype, Newell and Hymowitz induced flowering by grafting. They grafted it onto *G. max* var. Wayne. The transplanted form PI 393567 plants produced both tiny flowers in leaf nodes and racemes. It is a matter open to discussion if the observed forms really do not possess the ability to form recemes, or if this is a specific reaction to conditions entirely different from natural ones. Among the collected 45 forms, 25 produced racemes. The first raceme in *G. tomentella* forms was noted at 5.5 to 13.3 nodes. A somewhat smaller variance range for this trait was found for *G. tabacina* forms — 3.5 to 7.6 (Table 4).

A wide range of interspecific variability was found for raceme length and number of flowers per raceme. In *G. tabacina* forms, the raceme length ranged from 5.6 to 14.8 cm, and the number of flowers per raceme from 6.1 to 13.1. In *G. tomentella*, the raceme length was from 4.8 to 11.6 cm, the number of flowers per raceme, 4.9-9.7 (Table 4). The small values of the variance coefficient for the node from which the first raceme grew, for raceme length and number of flowers per raceme, indicate that the genotype greatly influences these traits. The following forms may be interesting for work with crosses: from the species *G. tabacina* — Chinchilla, 1077, PI 139232, WIR 36 7157, from *G. tomentella* — PI 245332 and *G. falcata*, represented in this experiment by one form which produced long (av. 21.5 cm), stiff racemes with about 15.0 flowers (Table 4).

The morphology of the flowers in the racemes of the studied species seems to favor attempts at crossing. The flowers were large (to 1 cm), with a highly developed perianth, colored from light-pink through amaranth to violet (Fig. 2). During blooming, the petals unfolded, the vexillum was bent far back. The structural properties of the raceme flower suggest that there is a tendency to allogamy in the subgenus *Glycine*. Additional tiny, colored flowers in the racemes of Miles form plants were observed. The number of these flowers approached 45. The structure of flowers of wild species was similar to the domesticated soybean flowers from forms which bloom chasmogamically. Unfortunately a large number of flowers in the racemes of wild species abscised. Abcission of flowers was also observed in *G. max*. Plants from these species loose from 20 to 70% of their flowers depending on the variety, environmental factors, humidity, temperature. Albernethy et al. (1977) concluded that fertility disorders do not seem to play a greater role in flower abscission in *Glycine max*.

In our study, the effectiveness of blooming expressed as the ratio of formed pods to the number of flowers depended on the flower structure and course of pollination. The effectiveness of pollination and fertilization of cleistogamic flowers was very high. Pod formation was very good. Studies done using the fluorescence method showed thick bundles of pollen

Table 4

Differences in morphological traits associated with the flowering and pod formation in racemes between form of subgenus *Glycine*

Form	Number of the first node with raceme				Length of raceme				Number of flowers per raceme				Percentage of pods setting			
	1981	1982	$\bar{x}$	V%	1981	1982	$\bar{x}$	V%	1981	1982	$\bar{x}$	V%	1981	1982	$\bar{x}$	V%
Glycine canescens																
401	7.6	9.4	8.5	10.6	5.5	6.8	6.2	10.5	6.4	9.8	8.1	20.9	9.3	0	9.6	70.7
Glycine clandestina																
436	6.4	6.6	6.5	1.5	4.7	3.9	4.3	9.3	6.8	6.2	6.5	4.6	14.5	10.0	12.2	18.4
449	9.2	8.5	8.8	4.2	5.9	6.0	6.0	0.8	7.2	10.0	8.6	16.3	14.4	5.8	10.1	42.6
Glycine falcata																
PI 233139	3.6	4.0	3.8	5.3	20.0	23.0	21.5	6.9	16.0	14.0	15.0	6.7	0	0	0	0
Glycine tabacina																
Chinchilla	9.0	6.2	7.6	18.4	16.8	12.8	14.8	13.5	12.4	11.0	11.7	5.9	0	9.0	4.5	70.7
Giken	7.0	3.8	5.4	29.6	14.2	10.8	12.5	13.6	10.2	8.2	9.2	10.9	3.5	0	1.7	70.7
Helidon	5.8	4.0	4.9	18.4	14.8	9.4	12.1	22.3	13.0	10.6	11.8	10.2	4.6	1.8	3.2	43.7
Hutton	8.4	4.8	6.6	27.3	11.7	6.6	9.2	27.2	10.2	6.2	8.2	24.4	1.7	0	0.8	70.7
Miyakojima tsurumame	5.6	4.6	5.1	9.8	6.6	4.6	5.6	17.8	8.8	6.3	7.6	16.4	2.3	0	1.2	70.0
Miles	5.4	6.4	5.9	8.3	12.0	10.0	11.0	9.1	13.0	10.4	11.7	11.1	3.2	6.2	4.7	31.9
Taichung 4	6.4	4.5	5.5	17.3	10.8	8.1	9.5	14.7	7.6	5.5	6.6	15.9	0	0	0	0
1077	3.8	4.0	3.9	2.6	12.4	8.8	10.6	16.9	14.2	12.0	13.1	8.4	2.8	0	1.4	70.7
1258	3.8	4.2	4.0	5.0	7.9	6.5	7.2	9.7	10.2	8.7	9.5	7.9	13.7	0	6.8	70.7
PI 193232	3.8	2.2	3.5	8.6	14.2	13.7	13.9	2.5	11.0	11.4	11.2	1.8	1.8	0	0.9	70.7
PI 248253	6.6	4.2	2.4	22.2	10.4	8.8	9.6	8.3	8.0	5.6	6.8	18.4	2.5	0	1.2	70.7
PI 272099	6.8	4.8	5.8	17.2	11.2	13.8	12.5	10.4	8.0	10.0	9.0	11.1	8.9	0	4.4	70.7
PI 319697	6.4	4.5	5.5	18.2	8.2	11.4	9.8	16.3	6.8	10.2	8.1	25.9	0	0	0	0
PI 37870	4.6	5.2	4.9	6.1	9.3	8.0	8.6	6.9	8.4	10.0	9.2	8.7	7.1	0	3.6	70.7
WIR 367157	3.6	3.8	3.7	2.7	16.3	12.4	14.4	13.9	13.2	11.2	12.2	8.2	6.0	0	3.0	70.7
WIR 367178	6.4	5.3	5.9	9.3	8.0	8.0	8.0	0	6.8	5.4	6.1	11.5	0	0	0	0
Glycine tomentella																
Eskdale	6.2	5.0	5.6	10.7	5.5	6.0	5.7	4.4	7.2	7.6	7.4	2.7	0	0	0	0
Inverale	7.4	6.6	7.0	5.7	8.1	8.2	8.1	0.6	6.4	8.6	7.5	14.7	12.5	0	6.2	70.7
486	17.4	12.0	13.3	9.8	5.0	4.7	4.8	3.1	5.1	4.7	4.9	4.1	0	0	0	0
PI 245332	4.6	6.4	5.5	13.4	10.7	10.3	10.6	0.6	10.8	8.6	9.7	11.3	0	0	0	0
PI 319697	5.6	6.6	6.1	8.2	12.3	9.7	10.5	17.1	8.6	7.0	7.8	10.2	7.0	0	3.5	70.7
WIR 367 177	9.0	8.4	8.7	3.4	10.5	12.6	11.6	8.6	8.2	7.0	7.6	7.9	0	0	0	0

$\bar{x}$ — average values of traits, V — variance coefficient of analysed traits.

tubes in the pistil styles growing through the pistil and penetrating into the ovaries of all of the analysed forms (Fig. 3). In the chasmogamic raceme flowers, however, pollen grains were seen germinating in the anthers, which remained closed. Sporadically, single germinating pollen tubes were seen in pistil styles. It seems that disturbances in the process of pollination in the raceme flowers may be one of the important reasons for flower abscission and low effectiveness of blooming in wild *Glycine* species. Plants from *G. tabacina* forms produced up to 6.8% pods, *G. tomentella* forms — 6.2%, *G. clandestina* — up to 12.9%. In addition, two year long observations showed that pod formation in racemes is a highly variable trait (Table 4). Intense chasmogamic flower abscission may cause serious problems in the cases where wild forms of the subgenus *Glycine* are used for wide crosses. The process of pod formation would then also be influenced by the effect of isolation barriers for generative reproduction which takes place in this type of crosses. It seems that it is worth undertaking studies on finding factors and methods leading to increasing the effectiveness of flowering in racemes of wild *Glycine* forms.

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Obserwacje biologii kwitnienia dzikich form z podrodzaju Glycine

Streszczenie

Od 1977 roku gromadzono i namnażano formy dzikie z podrodzaju *Glycine*. Ogółem zgromadzono 45 form. Przeprowadzono obserwacje zakresu zmienności cech związanych z biologią i efektywnością kwitnienia. Wykonano badania procesów zapylania i kiełkowania łagiewek pyłkowych *in vivo* w kwiatach kleistogamicznych i chasmogamicznych. W wyniku tych analiz stwierdzono, że zjawisko bardzo słabego wiązania strąków w kwiatostanach groniastych może być związane z zaburzeniami w procesie zapylenia.