Studies on the Pollen-Tubes.

II. The dependence between the potency of the pollen-tube growth in foreign styles and the thickness of the pollen-tubes and chromosome number.

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IN investigating the problem of the interspecific hybrids in *Nicotiana*, we stated that the following conditions are necessary for the successful production of the hybrids: (1) germination of the pollen of the paternal plant on the stigma of the maternal plant, (2) a necessary growth potency of the pollen-tubes in order to reach the ovary and penetrate into the micropyle of the ovule, (3) the occurrence of fertilization, (4) a satisfactory growth of the hybrid embryos, (5) germination of the seeds produced following hybridization, (6) surviving of the seedlings during the cotyledon stage and further development until maturity.

Previous investigations (Kostoff 1930, and Prokofieva 1934) showed that a definite dependence exists between the growth potency of the pollen-tubes and the length of the styles of the species from which the pollen originates. The longer the styles the plant has the greater growth-potency the pollen-tubes possess. The length of the styles of the maternal species plays an important rôle too. The shorter the styles are (the shorter the way between the stigma and the ovary) the sooner the pollen-tubes reach the ovary. When the styles of the maternal species are considerably longer than those of the paternal one, the pollen-tubes usually do not succeed to reach the ovary and the hybridization fails.

Further investigations show that this is nót the single cause for the failure of the hybridization. The measurements of the pollen grains and the pollen-tubes we carried out lately show definitely that in many cases the nucleoplasmic ratio of the pollen and further of the pollen-tubes represents a regulating factor for the velocity of the pollen-tube growth too.

The data given in Table 1 show that species with larger chromosome numbers (given there) have larger pollen grains. The same dependence seems to exist between the number of the chromosomes and the thickness of the pollen-tubes as shown in Tables 2 and 3. This is true for the auto-tetraploid forms when compared with the diploid forms. It also seems to be true for the amphidiploids (tetraploids) in relation to their parents and for the very closely related species but obviously not for the far related species.

The pollen-tubes of the tetraploid tomato ($4n = 48$) do not reach the ovary of the diploid ones ($2n = 24$) while in the reverse cross, namely $2n \times 4n$, the pollen-tubes of

TABLE 1.

Species	Chromosomes ($2n$) in the soma	Diameter of the pollen grains in microns										
		20	23	25·7	28·6	31·5	34·3	37·2	40	42·9	45·8	48·6
Nicotiana rustica ..	48	..	..	..	..	1	25	89	63	22	..	..
N. paniculata	24	..	1	17	39	105	28	3	..	..	..	..
N. rupa, *i.e.*, an amphidiploid of *rustica* × *paniculata*	72	..	..	..	..	..	6	18	25	119	21	1
N. glauca	24	..	..	..	20	66	24	..	..	..	..	..
N. Langsdorffii ..	18	..	1	38	64	18	..	..	..	..	..	..
N. glauca × *Langsdorffii* amphidiploid ..	42	..	..	..	..	3	19	92	85	26	..	..
S. Lycopersicum (tomato) diploid ($2n$) ..	24	10	174	17	..	..	..	..	..	..	..	..
S. Lycopersicum—tetraploid ($4n$) ..	48	..	3	47	129	21	..	..	..	..	..	..

TABLE 2.

Species	The thickness of the pollen-tubes in microns															
	5·7	6·4	7·2	7·9	8·6	9·3	10	10·7	11·4	12·2	12·9	13·6	14·3	15·0	15·7	16·4
Nicotiana rupa ..	..	..	..	..	1	3	3	2	26	6	7	7	24	4	12	4
N. rustica ..	..	..	..	..	22	16	18	7	30	1	3	3	1	..	..	..
N. paniculata ..	2	3	8	14	50	16	7	1	2	..	..	..	..	..	..	..

TABLE 3.

Form	The thickness of the pollen-tubes in microns											
	47	52	57	62	67	72	77	82	87	92	97	Total number
Diploid tomato ..	1	81	62	15	3	1	..	..	..	..	..	163
Tetraploid tomato ..	..	..	..	1	..	42	48	31	37	18	3	180

$2n$-plants with n-chromosome number reach the ovaries of the $4n$-plants.

The pollen-tubes of *Nicotiana rupa*, an amphidiploid plant from the F_1 (*N. rustica*, $n = 24 \times$ *N. paniculata*, $n = 12$), reach the ovary of *Nicotiana rupa* and of *Nicotiana rustica*, but rarely of *N. paniculata*, while the pollen-tubes of *rustica* and *paniculata* reach easily the ovary of *N. rupa*.

The pollen-tubes of the amphidiploid plants of *N. glauca* (n=12) $\times$ *N. Langsdorffii* ($n = 9$) do not reach the ovary of *N. Langsdorffii*, while the pollen-tubes of *N. Langsdorffii* reach easily the ovary of the amphidiploid hybrid which has 42 somatic chromosomes. We must here point out that the length of the styles is in favour of the cross *N. Langsdorffii* × amphidiploid (*glauca* × *Langsdorffii*), though the pollen-tubes do not reach the ovary in this cross combination but in the reciprocal one. Consequently, the thickness of the pollen-tubes seems to be here also the responsible factor.

In order to be able to estimate correctly the importance of the chromosome number in the pollen-tube growth process following inter-specific crosses, the factor "length of the styles" must be eliminated. The investigations in *Triticum* inter-specific hybrids show that the crosses $4n \times 6n$ are more successful than the reciprocal $6n \times 4n$ (Literature, see in Katayama, 1933). There is not a definite rule in *Nicotiana* if we do not consider the length of the styles. Thus, for example, the pollen-tubes reach easier the ovary in the following cross combinations: *N. rustica* ($n = 24$) × *N. Tabacum* ($n = 24$), *N. rustica* × *N. paniculata* ($n = 12$), *N. Tabacum* × *N. sylvestris* (n=12), *N. Tabacum* × *N. glauca* ($n = 12$), *N. glauca* × *N. Langsdorffii* ($n = 9$), *N. paniculata* × *N. Tabacum*, *N. suaveolens* ($n = 16$) × *N. Tabacum*, *N. glutinosa* (n=12) × *N. Tabacum*, *N. Langsdorffii* × *N. longiflora* ($n = 10$), etc., than in the reciprocal crosses. If we judge these examples as they are without consideration of the other factors involved in the pollen-tube growth, we must conclude that there is not any dependence between the number of the chromosomes and the growth of the pollen-tubes. Such a conclusion is undoubtedly wrong. In order to have really an unquestionable criteria of the significance of the chromosome number in the parental species for the velocity of the pollen-tube growth we must unconditionably consider the length of the styles of the species crossed, and which plant is used as maternal one. In other words, the factor "length of the styles" must be eliminated, and then the study of the crossability of the species with various chromosome numbers in relation to the velocity of the pollen-tube growth can be possible. For such a study we must take species that have approximately the same length of the styles, but different chromosome numbers.

The middle lengths of the styles of some *Nicotiana* species are given in Table 4. *N. Tabacum* var. *macrophylla* has a style of about 42 mm. *N. Sanderæ* of about 40 mm. The pollen-tubes of *N. Sanderæ* reach the ovary of *N. Tab. macrophylla*, while the pollen-tubes of *N. Tab. macrophylla* do not reach the ovary of *N. Sanderæ*. *N. Rusbyi* and *N. Tabacum* var. *sanguinea* have approximately the same length of the styles, though different chromosome numbers, and the pollen-tubes of *N. Rusbyi* reach the ovary of *N. Tabacum sanguinea*, while those of the latter do not reach the ovary of the former. *N. Langsdorffii* has even shorter style than *N. Rusbyi*, *N. paniculata*, *N. glauca*, *N. Tabacum* and *N. glutinosa*, but it has $n=9$, *i.e.*, less than any one of these species and its pollen-tubes reach the ovaries of all these species, while in the reverse crosses only the pollen-tubes of *glauca* may in exceptional cases reach the ovary of *N. Langsdorffii*. *Langsdorffii* pollen-tubes may sometimes reach even the ovary of *N. longiflora*, a species with the longest styles.

TABLE 4.

Species	Somatic chromosome number	Approximate length of the styles in mm.
N. longiflora[1]	20	ca. 85
N. Tabacum var. *macrophylla*	48	ca. 42
,, ,, ,, *sanguinea*	48	ca. 34
N. Langsdorffii	18	ca. 17·5
N. glauca	24	ca. 25
N. paniculata	24	ca. 24
N. glutinosa	24	ca. 20
N. Sanderæ (pink)	18	ca. 40
N. Rusbyi	24	ca. 33
Petunia violacea	14	ca. 7
N. rustica	48	ca. 10–12

[1] The environmental conditions and even the age of the plant influence somewhat the length of the styles.

Petunia violacea is a species very closely related to those of the genus *Nicotiana*. It has a very short style, though its pollen-tubes reach easily the ovaries of *N. Langsdorffii*, *N. Rusbyi*, *N. paniculata*, *N. glutinosa*, *N. glauca*, and even that of *N. Tabacum*, while the pollen-tubes of all these *Nicotiana* species cannot reach the ovary of *Petunia*. It is probably because *Petunia* has only $n=7$ and $2n=14$.

From the data here reported we can conclude that the thickness of the pollen-tubes plays an important rôle in the species and variety crosses; the thicker pollen-tubes grow slower than the thinner ones. When we eliminate the factor "length of the styles" by considering only such species that have approximately the same length of the styles one sees the following tendency: the pollen-tubes, originating from species that have larger chromosome number, grow slower in the styles of species having smaller chromosome number, than those originating from species with smaller chromosome number, when growing in styles of species with larger chromosome number. Consequently, a species cross $A \times B$ would be more successful than its reciprocal $B \times A$, when A has larger chromosome number than B, because the pollen-tubes of B reach easier the ovary of A than in the reverse cross.

Literature.

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