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# INFLUENCE OF POLLEN HEATING ON THE QUALITY OF RESULTING SPOROPHYTE GENERATION IN SUNFLOWER

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SUMMARY

Influence of heating a heterogeneous pollen population on some quantitative characters of the resulting sporophytic generation was studied in sunflower. Pollen of interspecific hybrids *H. annuus* cv. Lider x *H. argophyllus* and *H. annuus* cv. Yubileiny x *H. bolanderi* was treated with the temperature of 40-60°C during 1-3 hours, then used for backcrossing. Heating pollen at 60°C for 3 hours was most effective. This treatment significantly increased the frequency of taller plants as well as the portion of genotypes with longer emergence-flowering stage and minimum number of branches. Taking into consideration that the parental forms of the used hybrids differed in these traits, the conclusion can be drawn that heat treatment selectively affected pollen grains favoring gametes of a cultivated genotype.

**Key words: Pollen, heat treatment, sporophytic performance, interspecific hybrids, sunflower**

## INTRODUCTION

The data accumulated on the genetics of microgametophytes allow a conclusion that the significant portion of genes of the angiosperm plants is expressed at both diploid and haploid stages of life cycle (Ottaviano and Mulcahy, 1989). In this connection, selection in the male gametophytic generation (pollen selection) is recently considered as an effective breeding technique, capable of readily causing essential changes in the structure of sporophyte populations (Ottaviano *et al.*, 1990 and Lyakh, 1995). Thus, for a number of crops it was shown that pollen heating before pollination resulted in a raise of drought tolerance of the subsequent sporophytic generation (Rodriguez-Garay and Barrow, 1988; Lyakh and Soroka, 1993, Mishchenko and Lyakh, 1997).

In comparison with sporophytes, haploid generation of angiosperm plants has some advantages such as the significant size of the male gametophyte population

and accessibility for routine laboratory analyses. Due to the fact that recessive alleles in the haploid genome cannot be hidden inside heterozygotes and are exhibited phenotypically, they are available at any moment to selection pressure.

The aim of the given paper was to study the influence of the treatment of pollen of $F_1$ sunflower hybrids with high temperature on the structure of sporophytic populations raised after pollination with the treated pollen.

## MATERIALS AND METHODS

$F_1$ interspecific hybrids *H. annuus* cv. Yubileiny x *H. bolanderi*, *H. annuus* cv. Lider x *H. argophyllus* were used as experimental material.

The parental components of the hybrids differed in a number of quantitative characteristics. Plants of the varieties had a height of 200-220 cm, emergence - flowering stage lasting on average 60 days, no lateral branches. The plants of the "wild types" were not so tall (in a greenhouse their height was on average 100-150 cm), they entered flowering 10-15 days earlier than the varieties and had a large number of lateral branches.

For making a selection in $F_1$, measured lots of 0.5 $cm^3$ of freshly collected pollen were heated at the temperature of 40°C during 3 hours, at 60°C during 1 and 3 hours for Yubileiny x *H. bolanderi*, and at 60°C during 3 hours for Lider x *H. argophyllus* and then used for pollination of parental plants to derive backcrosses. Freshly collected pollen was used for control pollinations. Three inflorescences representing three replications were pollinated for each treatment of Yubileiny x *H. bolanderi*, and five inflorescences for Lider x *H. argophyllus*. Seeds resulting from each inflorescence were randomly sown and grown under field conditions. Influence of the various treatments of $F_1$ pollen on the quality of segregating sporophytic $BC_1$ populations was estimated by recording plant height, duration of emergence-flowering phase and number of lateral branches, recognizing that the parental forms of the hybrids contrasted for the characters studied.

Reliability of deviations of experimental data from the control was evaluated by standard t- and F-criteria using MSTAT-C software package (MSTAT-C, 1991).

## RESULTS AND DISCUSSION

The data listed in Table 1 show that heating of pollen of $F_1$ plants at the temperature of 60°C during 3 hours exerted the most notable influence on the structure of segregating $BC_1$ populations. These temperature conditions resulted in essential changes in plant height, duration of the emergence-flowering phase, and the number of lateral branches developed by plants.

In Yubileiny x (Yubileiny x *H. bolanderi*) $BC_1$ cross, the average plant height after pollen treatment at 60°C during 3 hours was considerably larger than in the

Table 1: Influence of pollen treatment on segregation of plants in $BC_1$ populations for some quantitative traits, %

| Treatment | Height (cm) | | Emergence-flowering | | Lateral branches | |
|---|---|---|---|---|---|---|
| | <200 | >200 | <55 days | >55 days | present | absent |
| | $BC_1$ Yubileiny x (Yubileiny x *H. bolanderi*) | | | | | |
| Control | 75.8 | 24.2 | 59.1 | 40.9 | 77.4 | 22.6 |
| 40°C/3hr | 70.8 | 29.2 | 61.5 | 38.5 | 72.3 | 27.7 |
| 60°C/1hr | 71.6 | 28.4 | 51.7* | 48.3* | 72.9 | 27.1 |
| 60°C/3hr | 53.5** | 46.5** | 25.6*** | 74.4*** | 32.6*** | 67.4*** |
| | $BC_1$ Lider x (Lider x *H. argophyllus*) | | | | | |
| Control | 74.3 | 25.7 | 58.6 | 41.4 | 28.6 | 71.4 |
| 60°C/3 hr | 52.8* | 47.2* | 44.4* | 55.6* | 19.4 | 80.6 |

All data represent averages based on observations from three replications for $BC_1$ Yubileiny x (Yubileiny x *H. bolanderi*) and from five replications for $BC_1$ Lider x (Lider x *H. argophyllus*).
*, **, *** - the differences from the control are significant at $P < 0.05$, 0.01, and 0.001, respectively.

control (196.5±3.8 cm as compared with 182.9±1.6 cm). The same experimental population in comparison with the control possessed almost two times more plants with a height exceeding 200 cm. Similar changes were also obtained while analyzing sporophytic populations of Lider x (Lider x *H. argophyllus*). In the given case the average height of plants in the control was 182.3m, while in the experimental population it was 194.6±5.0 cm, percent of tall (>200 cm) plants being 25.7 and 47.2% respectively. It is evident that the frequency of plants of "cultivated type" was increased in the experimental variants, while the frequency of plants of the "wild type" decreased proportionally.

The analysis of $BC_1$ populations for the duration of the emergence-flowering phase has shown that the 3-hr heating of pollen at 60°C resulted in an increase of the late-flowering plant portion. In Yubileiny x (Yubileiny x *H. bolanderi*) cross, after pollination with treated pollen there were about 75% of plants with "emergence-flowering" phase of more than 55 days that exceeded almost twice the given index in the control. Similarly, the experimental population with *H. argophyllus* had significantly more late-flowering plants compared with the control. Taking into account that longer emergence - flowering stage is characteristic of the varieties used for crossings, it is possible to assume that pollen heating resulted in an increase of the cultivated type plant percentage.

Segregating populations were also compared for development of lateral branches. In $BC_1$ Yubileiny x (Yubileiny x *H. bolanderi*) after pollination with pollen heated at the temperature of 60°C during 3 hours, the proportion of plants lacking lateral branches has increased more than three times in comparison with the control and amounted to 67.4 %. Experimental $BC_1$ Lider x (Lider x *H. argophyllus*) population also tended to comprise a higher portion of plants without lateral branches than the control.

While evaluating the resulting $BC_1$ populations for branching plant percent, we also estimated an average number of lateral branches per plant as well as a degree

of the trait variability (Table 2). Plant branching after pollination with heated pollen has considerably changed. Thus, in the Yubileiny x (Yubileiny x *H. bolanderi*) experimental population (60°C/3hr), the average number of lateral branches per plant in comparison with the control has decreased two and a half times, and in the cross with *H. argophyllus* - more than three times. Besides, as it is evident from the table, in the control of $BC_1$ Yubileiny x (Yubileiny x *H. bolanderi*) there were plants with more than 30 lateral branches whereas in the experimental population not more than 15 branches per plant were found. Similar changes took place in the $BC_1$ Lider x (Lider x *H. argophyllus*). Notable decrease of variance in the treatments also indicates the change of population structure after gametophytic selection. Prevalence of non-branching and weakly branching plants in the $BC_1$ experimental populations confirms again that heat treatment of pollen under conditions of 60°C/3 hr resulted in an increase of the cultivated type plant portion.

Table 2: Amount of lateral branches per plant in $BC_1$ populations after pollen treatment

| Treatment | Average number | Maximum number | Variance |
|---|---|---|---|
| | $BC_1$ Yubileiny x (Yubileiny x *H. bolanderi*) | | |
| Control | 5.0 | 32 | 23.7 |
| 40°C/3hr | 5.4 | 18 | 27.2 |
| 60°C/1hr | 5.1 | 23 | 22.5 |
| 60°C/3hr | 2.0*** | 15 | 14.8* |
| | $BC_1$ Lider x (Lider x *H. argophyllus*) | | |
| Control | 0.7 | 7 | 1.8 |
| 60°C/3hr | 0.2** | 1 | 0.2** |

Average number and variance represent averages based on observations from three replications for $BC_1$ Yubileiny x (Yubileiny x *H. bolanderi*) and from five replications for $BC_1$ Lider x (Lider x *H. argophyllus*).
*, **, *** - the differences from the control are significant at $P < 0.05$, 0.01 and 0.001, respectively

The results of the research showed that pollen heat treatment at 40°C/3 hr and 60°C/1 hr did not practically cause selective elimination of microgametophytes in the hybrids studied. Higher temperature background (60°C/3hr) rendered significant selective pressure, however, resulting in the preference of pollen grains mainly with genotypes close to wild type, which was evident from a substantial increase of plants with cultural variety characteristics in the $BC_1$ experimental populations. Similar selective elimination of pollen grains at 60°C was earlier found in tomato (Kravchenko *et al.*, 1988).

The genes confering development of the above mentioned quantitative traits of sporophyte are unlikely expressed at the stage of pollen grain. The genes that confer resistance of gametophytic generation to high temperature stress are probably linked in the sunflower to genes conferring quantitative characteristics such as height, flowering date, and lateral branch development at the sporophytic stage of plant development. Due to this linkage pollen treatment with high temperature lead to the change in the sporophytic population structure.

Pollen selection for tolerance not only to various abiotic stresses is currently considered as one of the effective methods capable of readily ensuring essential changes in genetic structure of populations. Several examples are known of effective microgametophytic selection for resistance to some fungal deseases (Balashova *et al.*, 1992). Besides, different authors both recently and earlier noted that selection in the male gametophytic generation for pollen competitive ability, basic component of which is considered to be pollen tube growth, can essentially influence progeny performance (Ottaviano *et al.*, 1990 and Bjorkman, 1995).

The obtained results are of interest in practical work with interspecific sunflower hybrids. For example, one of common breeding problems is to fix in consequent generations only defined characters of a wild species and reduce to a minimum the development of others. One of the undesirable traits, for example, is the development of many lateral branches per plant. Therefore, selection of microgametophytes, resistant to high temperatures, can be used to diminish the amount of wild type plants in resulting populations.

Thus, the research revealed that pollen heating can serve as a way to modify the structure of the sporophytic population in sunflower.

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## INFLUENCIA DE CALENTAMIENTO DE POLEN SOBRE LA CALIDAD DE LAS RESULTADAS GENERACIONES ESPOROFITICAS EN GIRASOL

RESUMEN

La influencia de calentamiento de populaciones heterogeneas de gametofitos sobre la estructura de populaciones esporofiticas por algunas caracteristicas cuantitativas fue estudiada en girasol. Polen de los hibridos interespecificos *H. annuus* (variedad Lider) x *H. argophyllus* y *H. annuus* (variedad Yubileiny) x *H. bolanderi* fue tratado con la temperatura de 40-60°C durante 1-3 horas, despues el fue utilizado para obtener $BC_1$. El calentamiento de polen a temperatura 600°C durante 3 horas fue el mas efectivo. Este tratamiento de polen aumentaba considerablemente la frequencia de plantas mas altas tambien como la porcion de los genotipos con el periodo "germenes-floracion" mas largo y la cantidad de retonos mas minima. Tomando en consideracion que componentes paternales de los hibridos utilizados se han distinguido entre si por estas propiedades, se puede sacar conclucion que tratamiento de polen con la temperatura elevada ha influido selectivomente sobre granos de polen favoreciendo a los gametos de genotipo de cultivo.

## EFFET DU TRAITEMENT THERMIQUE DU POLLEN DE TOURNESOL SUR LA QUALITÉ DU SOROPHYTE DE LA GÉNÉRATION SUIVANTE

RESUME

L'effet du chauffage d'une population hétérogène de pollen sur la structure du sporophyte à la génération suivante a été étudié chez le tournesol pour des caractères quantitatifs. Le pollen d'hybrides interspécifiques *H. annuus* cv Lider x *H. argophyllus* et *H. annuus* cv. Yubileiny x *H. bolanderi* a été traité à la température de 40-60°C durant 1-3 heures, puis utilisé en rétrocroisement. Le chauffage du pollen à 60 °C, pendant 3 heures fut le plus efficace. Ce traitement a augmenté significativement la fréquence des plantes de taille élevée ainsi que le taux de génotypes à phase levée-floraison plus longue ou à ramification réduite. Compte tenu de la diversité de ces caractères chez les parents des hybrides, on peut en conclure que le traitement thermique du pollen a affecté sélectivement les grains de pollen en favorisant les gamètes de type cultivé.