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*Original Article*

# Pollen Quality as a Criterion for Selection of Tomato Genotypes Resistant to Stress Abiotic Factors

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***Abstract*** *- The results of a study of pollen quality, one of the most important factors of reproductive biology, in multi-marker tomato lines differing in the type of plant growth ($sp^+$, $sp^\pm$, sp), the length of internodes and the intensity of the formation of lateral shoots (ls, br), shape, weight, and color are shown and marketability of fruits (nor, rin, alc, u, r, t, bk, ck, j, j-2). The research was carried out in 2019-2021. Pronounced genotypic differences between the lines were established in the nature of the manifestation of traits: fertility, pollen viability, length of pollen tubes and morphological characteristics of pollen grains, both depending on the level of inflorescence position on the plant and the influence of stressful abiotic factors (high temperature, drought). It has been shown that different traits of one genotype vary unequally, having certain limits which indicate their genetic determination. In the pollen populations of lines 196, 197, 234, 237, and 241, carriers of two mutant marker genes (ls and br), the number of pollen grains with morphological disturbances in their integrity is greater. Lines 110, 164, and 178 were also identified, with a stable manifestation of all the studied characters. The share of influence of genotype, stress factors and their interaction in the overall variability of traits on the nature of their manifestation is shown, which indicates the effectiveness of using pollen as a criterion for assessing, identifying and selecting genotypes resistant to abiotic stress factors.*

***Keywords*** *- Lines, Mutant genes, Male gametophyte, Resistance, Tomato.*

## 1. Introduction

The susceptibility of plants to various unfavorable environmental factors: high and low temperatures, drought, soil moisture deficiency, sudden changes in night and day temperatures, soil salinity, etc., leads to intensified genetic erosion and, as a consequence, to a significant decrease in the potential productivity of not only tomatoes but also other crops (Kang, 2004; Fentic, 2017; Alagna et al., 2020). Various authors state that environmental sustainability in the plant world is one of the scarcest economically valuable traits (Zhuchenko, 2004; Udovenko, 2011; Fentic, 2017; Alagna F. et al., 2020). It is the narrow limits of genetic diversity of environmental resistance, for example, to temperature stress, according to Zhuchenko (2012), that was the main reason that over the past 50 years, a significant increase in the upper level of tolerance to these factors in major crops had not been achieved. Consequently, great attention in the breeding system of tomatoes and other crops should be paid to the search, preservation, identification and use of appropriate gene sources. The creation of tomato varieties and hybrids that are resistant to abiotic stress factors through selection is one of the main conditions for the realization of their genetically inherent potential productivity. The difficulty in solving this problem lies in the fact that it is very difficult to determine the stability of the selected material under natural conditions. It is necessary that an unfavorable factor (high, low temperatures, drought, etc.) manifest itself quite severely precisely during the period of plant development when they are most sensitive to it. Conducting such assessments is a rather labor-intensive and expensive procedure in field conditions. According to Pfahler (1982), one of these stages of ontogenesis is the male gametophyte of plants, the role of which in the process of adaptive selection must be given special attention. This means that innovative methods of gamete analysis in vitro can come to the rescue, allowing you to quickly select genotypes resistant to unfavorable environmental factors. They began to be used quite actively in breeding practice (Mulcahy et al., 1982; Mascarenhas et al., 1982; Ottaviano E., 1985; Kravchenko et al., 1988; Kakani et al., 2012; Yeug-Kyung Chang at el, 2010; Lyakh et al., 2014; Singh et al., 2017; Makovei, 2018; Demurin at el., 2022). The selective influence of different factors on the morphological characteristics of pollen has been established, which allows them to be differentiated by phenotype (Matamoro-Vidal et al., 2012). The effectiveness of using pollen as a criterion for selecting genotypes resistant to moisture deficiency has been shown (Patil, B. at el., 2006). Pollen is also used to determine the most productive flowers in an inflorescence (Blackmore et al., 2007; Jung et al., 2009). The quality of pollen, being one of the most important factors of reproductive biology (Zhuchenko, 2012), determines the efficiency of both natural and artificial pollination processes, which in turn depend on the characteristics of pollen formation under the influence of certain environmental stress factors. The information presented shows that pollen grains' quality is influenced by many factors - high and low temperatures, soil salinity, plant damage by diseases and pests, and even the introduction of plants, which lead to disruption of physiological and biochemical processes. This, in turn, provokes abnormal development of pollen grains, a decrease in their fertility and, as a consequence, a decrease in reproductive potential, which is reflected in the level and quality of the resulting crop and seeds (Pressman E. 2002; Hsu S.-Y. et al. 2003; Reyes et al. al. 2007; Batygina et al. 2010). Many authors have identified a high negative impact on the quality of pollen and anthropogenic factors: the use of herbicides, soil salinity, high levels of ozone and carbon dioxide in the air, the acidity level of precipitation, and the accumulation of heavy metals in the soil.

This leads to an expansion of the range of anomalies of the male gametophyte at the stage of formation, affects the size and other morphological features of the pollen grain, the biochemical composition, which in turn affects the fertility and fertilizing ability of mature pollen (Aloni et al, 2001; Izmailov, 2002; Apel et al. 2004; Klimenko et al. 2005; Suzuki et al. 2006; Solntseva et al. 2010). Considering the increased sensitivity of male gametes to external factors, using pollen analysis, it is possible to obtain primary information about the response of genotypes to the action of a specific stress factor and to identify valuable gene sources at the early stages of the breeding process to create new, more resistant forms of tomato.

Assessing the level and identifying forms of tomato resistance to specific stress factors in natural conditions is complicated by the combination of the action of a complex of stress factors (Koshkin, 2010), which usually complicates or makes such testing in the field impossible. Therefore, to assess the resistance of plants, along with the action of natural factors, it is necessary to simulate them in laboratory conditions. Their advantage is the ability to regulate the level of severity of the action of the unfavorable factor for which selection is carried out (high, low temperatures, drought, etc.) and, thus, intensify the assessment and selection of the appropriate source material and thereby speed up the selection process (Makovei 2018).

Taking into account the above information, the purpose of the research was, using artificially created stress backgrounds, to study the nature of the manifestation of male gametophyte traits affecting the reproductive functions of plants - fertility and pollen viability, the length of pollen tubes, resistance to pollen germination and the length of pollen tubes, as well as the size of pollen grains depending on the genotype, the level of inflorescence position on the plant and the action of abiotic stress factors, in multi-marker tomato lines obtained from crossing forms of carriers of the mutant genes *ls* (*lateral suppressor*) and *br* (*brachytic*), the combined influence of which leads to morphological deviations in the structure of flowers or their individual elements.

## 2. Material and Methods

The object of research was 16 multi-marker tomato lines (L110, L164, L178, L181, L183, L188, L193, L195, L196, L198, L201, L204, L228, L234, L237, L241) with different combinations of economically valuable traits, differing in the type of plant growth ($sp^+$, $sp^\pm$, *sp*), the length of internodes and the intensity of the formation of lateral shoots (stepchildren), controlled by the *ls* and *br* genes, different in the structure of the reproductive organs, as well as shape, weight, color, marketability and quality of fruits (*nor, rin, alc, u, r, t, bk, ck, j, j-2* et al.). Plants were grown in summer greenhouses according to the scheme for lines with a determinate growth type: 5.5-6 plants/m$^2$, for lines with semi-determinate and indeterminate growth types: 4-4.5 plants/m$^2$.

Studied the morphological characteristics of pollen grains, the fertility and viability of pollen, resistance to germination and the ability of pollen grains germinated against the background of stress factors to form pollen tubes of sufficient length for fertilization. The nature of the manifestation of the indicated pollen characteristics was determined depending on the level of position of the inflorescence on the plant's main stem and its reaction to the action of high temperature and osmotic factors. For this purpose, pollen was collected separately from flowers of 1st and 2nd inflorescences (lower tier), 3-4 inflorescences (middle tier) and 5-6 inflorescences (upper tier). Pollen from 100 flowers in each research variant and along each line was isolated with a specialized vibrator (VB-93). The size (diameter of pollen grains) of freshly collected pollen (control) and after exposure to stress factors (experiment) was calculated by measuring them under a microscope (Zeiss type) using an eyepiece and an object micrometer (Pausheva, 1988).

Determine the quality of pollen and its resistance to stress factors (high temperature, drought), freshly collected pollen from each line was divided into 4 parts and used for experiments according to the scheme:

1. Determination of the ratio of sterile and fertile pollen grains in populations by staining it with acetocarmine (Pausheva, 1988). Fertile pollen grains become red after staining, while sterile pollen with other anomalies in development does not stain; 2. Assessment of the viability of freshly collected pollen (control), isolated from flowers of inflorescences of each line by germination on an artificial nutrient medium containing 15% sucrose and 0.006% boric acid in vitro for 3 hours at a temperature of 25˚C (Golubinsky, 1974). Germinated pollen grains were counted, and the length of pollen tubes was measured; 3. Heat resistance was tested by exposing freshly collected pollen to a temperature of 45˚C for 8 hours, followed by germination on a nutrient medium of the above composition for 3 hours (Makovei, 2018); 4. Drought resistance was determined by the ability of pollen to germinate and form pollen tubes of sufficient length for fertilization on a nutrient medium with a high concentration of sucrose (38%, simulating drought) with the addition of boric acid (0.006%)

when germinated *in vitro* for 5 hours (Makovei, 2018). In each research option, at least 500 pollen grains were considered and analyzed according to the nature of their germination and ability to form long pollen tubes. The length of the tubes was measured in divisions of the eyepiece micrometre (d.e.m). Pollen grains in which the length of the pollen tubes was at least three times the diameter of the pollen grain were considered germinated. Resistance was determined using the resistance coefficient (Ignatova, Makovei, 2014), which combines two parameters in one indicator and gives a complete picture of the resistance of the genotype to a specific stress factor.

$$Kstr = \frac{Pex \times Lex}{Pc \times Lc} \times 100\%$$

Where $K_{str}$ is the stress resistance coefficient, expressed in %; *Pex* is the number of germinated pollen grains in the experiment, pcs.; *Lex* is the length of pollen tubes in the experiment (d.e.m.); *Pc* is the amount of sprouted pollen in the control pcs.; *Lc* is the length of pollen tubes in control (d.e.m.).

The share of the influence of the genotype, stress factors and their interaction on the character of the manifestation of traits was determined in a dispersion complex using the *Statistica 7* program.

## 3. Discussions

Marker characteristics (*ssp, sp,* $sp^{+}$, $sp^{\pm}$, *br, ls, pis, nor, rin, u, gs. j, j-2*), the carriers of which are the lines we created (Table 1), are easily identified in open and protected soil at different stages of plant growth and development. A possible limitation of their use in breeding programs may be the quality of their pollen since they are obtained from crossing forms of carriers of the *ls* and *br* genes, the presence of which in one genome causes significant morphological disturbances in the structure of the flower or its individual elements (Makovei, 2022). Consequently, their use in heterotic selection, especially as paternal components, requires information about the quality and quantity of formed pollen in inflorescence flowers, depending on the characteristics of the genotype and the influence of other factors. In this regard, fertility, pollen viability, morphological characteristics of pollen grains and the variability of these characteristics were studied depending on the level of inflorescence initiation on the plant and the effect of high temperature.

### *3.1. Morphobiological Characteristics of Lines*

Table 1 presents the results (average for 2019-2021) on the nature of the manifestation of the main selection-valuable traits. The presence of genes that control different types of plant growth (*sp, sp+, sp*±), high marketability of fruits (*rin, nor, alc*), limited formation of lateral shoots (*ls*), length of internodes (*br*) and the stable nature of their manifestation determine the value of these lines for the creation heterotic hybrids of industrial type.

Accounting and analysis of the characteristics of the manifestation of the main economically valuable traits in populations of generations of offspring lines ($F_4$-$F_6$) in three different complex climatic conditions of the year (2019-2021) revealed homogeneity and stability in the nature of the manifestation of traits controlled by mutant genes.

Lines 110, 183 and 188 are early ripening (96-103 days) with a main stem height of 50-65 cm. Others - 164, 178, 181 and 195 belong to the group of mid-early and mid-ripening forms of tomato (105-114 days) with a plant height of 70-98 cm. This group of lines was isolated from populations of hybrid combinations obtained using the mutant form Mo 443 (*ls*) and have a super determinate and determinate growth type. The plants are distinguished by a weak and medium degree of formation of lateral shoots, from 2.9 to 4.2 per 7.1 and 10.4 internodes of the plant's main stem. The average length of internodes for this group of lines is from 6.5 to 9.0 cm, which determines the close arrangement of inflorescences, their greater number on the main stem of the plant and the total number of fruits on them (Table 1). The stable nature of the manifestation of traits and their original combination presuppose their active inclusion in breeding programs for producing high-yielding and early-ripening varieties and determinate $F_1$ tomato hybrids.

Of particular value in the breeding plan for the creation of tall heterotic hybrids is the second group of multi-marker lines: 196, 197, 198, 204, 205, 228, 234, 237 and 241, obtained from splitting populations of hybrid combinations from crossing with the semi-cultivated mutant line 11069, which is the carrier of two genes (*ls, br*) (Table 1). They belong to the mid-early ripeness group (101-110 days) and have semi-determinate and indeterminate growth types. The length of the main stem of plants is from 150 to 250 cm, on which a limited number of reduced lateral shoots (stepchildren) are formed during the active growing season.

The number of stepsons on the main stem of a plant, depending on the genotypic characteristics of the line, ranges from 3.1 to 7.2 pieces. At 21.2-32.5 nodes, internodes are short and medium length (from 5.3 to 12.4 cm) (Table 1). The plant habit is characterized by an erect–semi-erect stem, with an optimal ratio of leaf mass and fruits. The color of the leaves is dark green, with different leaf shapes: ovate, elongated-ovate, and broadly lanceolate. Pronounced differences between the lines occur in the shape and morphological characteristics of the flowers: with large and small normally developed flowers; with a full corolla, but petals fused into a tube; with some deformations of the stamen column and the pistil protruding beyond the stamen column to varying degrees.

Line 196 is distinguished by an original combination of characteristics: sterility, potato leaf type, yellow-orange fruits, and semi-determinant growth type. With minor modifications, it can be successfully used as a mother component for creating heterotic $F_1$ hybrids on a sterile basis.

### 3.2. A Cytological Study and Analysis of Pollen Fertility

*A cytological study and analysis of pollen fertility* of each line, including relative to the level of inflorescence position on the main stem of the plant, revealed pronounced differences between them not only in the coloration of pollen grains but also in morphological heterogeneity in the form of underdeveloped, different in size with impaired integrity and other anomalies associated with the influence of mutant genes. Normally, tomato pollen fertility is 90-100%.

It was established that in the populations of pollen lines 110, 164, 178, 181, 183, 188 and 195, isolated from hybrid combinations with the mutant form Mo 443 (ls), sterile and with different morphological deviations of pollen grains, there is a small amount (from 2 to 16, 3%), the rest were well stained, which characterizes their high fertility (from 83.7 to 97.4%). Whereas in pollen populations of lines 196, 197, 198, 204, 205, 228, 234, 237 and 241, obtained from combinations from crossing with line 11069 (ls, br), heterogeneity is higher. There are quite a lot of pollen grains with morphological disturbances of varying degrees of severity (Table 2).

Differences are also expressed between the lines, including depending on the level of position of the inflorescence on the stem of the plant from the flowers from which pollen was isolated (Table 2). The highest heterogeneity was observed in pollen populations of lines 196, 197, 234 and 237, which respectively have low fertility: 43.4%, 50.8%, 50.3% and 54.2%. The rest of the pollen was not stained, which indicates their sterility or they are at different stages of development due to the negative influence of the mutant ls and br genes.

In these lines, a decrease in the fertility rate of pollen obtained from flowers of inflorescences of a higher level on the plant (5-6 inflorescences) was also noted. Probably, critical temperature regimes and, as a rule, moisture deficiency, under which the formation of pollen in the flowers of inflorescences of the upper tiers of the plant falls, in combination with the negative influence of the *ls* and *br* genes, leads to an increase in the proportion of morphological disorders of pollen grains (Table 2).

**Table 1. Characteristics of new multi-marker tomato lines (2019-2021)**

| Lines no. | Origin of the line | Genotype | Number of internodes on the plant, pcs. | Average length of internodes, cm | Number of laterals shoots per plant, pcs. | Number of inflorescences on a plant, pcs. | Total yield, kg/m$^2$ | Marketability of fruits,% | Fruit weight, g |
|---|---|---|---|---|---|---|---|---|---|
| **110** | **Mo443 x Fakel** | *ssp, u, j, ls, br* | 7.1 | 6,5 | 3,4 | 4,5 | 8,4 | 90,3 | 106.4 |
| **164** | **Mo443xMaKrista** | *sp, u, ls, j-2* | 10.1 | 8,5 | 3.1 | 5.4 | 11.6 | 97.7 | 72.1 |
| **178** | **Mo443xMilOranj** | *sp, u, t, ls, f* | 9.0 | 9.0 | 4.2 | 4.7 | 13.8 | 87.4 | 97.3 |
| **181** | **Mo443xMaKrista** | *sp, hp-2, j-2, u* | 10.3 | 8.1 | 3.2 | 4.9 | 12.6 | 94.6 | 88.2 |
| **183** | **Mo443xMaKrista** | *ssp, u, ls, j-2, nor* | 8.8 | 7.4 | 3.0 | 4.4 | 10,2 | 95.3 | 75.5 |
| **188** | **Mo443xMilOranj** | *ssp, ls, β* | 9.2 | 7.0 | 3.3 | 4.7 | 9.1 | 90.2 | 74.7 |
| **195** | **Mo443xMaKrista** | *sp, u, ls, nor* | 10.4 | 7.0 | 2.9 | 5.3 | 13.5 | 94.8 | 72.2 |
| **196** | **L11069xL2529** | *sp$^{\pm}$, ps, alc, c,ls,br* | 21.2 | 8.0 | 3.7 | 6.5 | 10.2 | 89.3 | 69.7 |
| **197** | **L11069 x L28** | *sp$^{+}$, ls, br, rin* | 32.5 | 6.8 | 3.3 | 11.3 | 15.7 | 94.3 | 68.1 |
| **198** | **L11069 x L28** | *sp$^{+}$,rin, ls, u* | 22.7 | 12.4 | 7.2 | 6.4 | 12.7 | 93.9 | 65.3 |
| **204** | **L11069 x L556** | *sp$^{\pm}$, ls, t, rin, bk* | 23.4 | 8.7 | 5.4 | 8.6 | 16.6 | 96.0 | 57.2 |
| **205** | **L11069 x L 556** | *sp$^{+}$, ls, u* | 22.4 | 11.9 | 5.8 | 7.1 | 13.1 | 95.1 | 54.1 |
| **228** | **L11069 x L111** | *sp$^{\pm}$, ls, u, ex* | 22.7 | 9.0 | 6.7 | 7.7 | 12.9 | 96.6 | 120.4 |
| **234** | **L11069 x L111** | *sp$^{\pm}$, u, nor, ls,br* | 21.1 | 6.5 | 3.1 | 8.1 | 11.8 | 94.7 | 74.2 |
| **237** | **L11069 x L828** | *sp$^{+}$, rin, ls, br* | 26.8 | 5.3 | 4.9 | 9.8 | 15.7 | 91.2 | 85.1 |
| **241** | **L11069 x L187** | *sp$^{+}$, ls, br, u, ck* | 28.1 | 6.4 | 4.5 | 9.0 | 18.3 | 92.5 | 114.3 |

**Table 2. Fertility and the proportion of morphological disturbances in the integrity of pollen grains in multi-marker tomato lines depending on the level of inflorescence position on the plant**

| Lines no | Pollen fertility depending on the level of inflorescence position on the plant, % | | | Average pollen fertility by genotype, % | Degree of damage to the integrity of pollen grains, % |
|---|---|---|---|---|---|
| | Inflorescences | | | | |
| | 1-2 | 3-4 | 5-6 | | |
| **110** ***ssp, u, j, ls, br)*** | 87.6 ± 2.79 | 90.8 ± 2.85 | 72.8 ± 2.81 | 83.7 ± 2.31 | 16.3 |
| **164** ***(sp, u, ls, j-2)*** | 96. 1± 1.86 | 96.3 ± 2.79 | 96.4 ± 2.36 | 96.1 ± 2.13 | 3.4 |
| **178** ***(sp, u, t, ls)*** | 89.9 ± 1.13 | 91.4 ± 2.35 | 89.2 ± 2.07 | 90.2 ± 2.23 | 9.8 |
| **181** ***(sp, hp-2, j-2, u)*** | 97.6 ± 2.77 | 92.4 ± 2.61 | 86.3 ± 1.74 | 92.1 ± 2.0 | 7.9 |
| **183** ***(sp, u, ls, j-2, nor)*** | 87.5 ± 1.14 | 83.6 ± 2.33 | 85.4 ± 1.83 | 85.5 ± 2.37 | 17.2 |
| **188** ***(ssp, u, ls)*** | 98.3 ± 1.92 | 87.7 ± 2.81 | 88.2 ± 2.64 | 91.4 ± 3.34 | 8.6 |
| **195** ***(sp, u, ls, nor)*** | 99.6 ± 2.65 | 96.4 ± 1.94 | 96.3 ± 2.85 | 97.4 ± 2.97 | 2.6 |
| **196** ***(sp$^{\pm}$, ps, alc, c, ls,br)*** | 41.8 ± 1.14 | 55.7 ± 1.27 | 32.8 ± 1.52 | 43.4 ± 1.78 | 56.6 |
| **197** ***(sp$^{+}$, ls, br, rin)*** | 53.6 ± 0.94 | 51.8 ± 1.72 | 47.1 ± 1.34 | 50.8 ± 2.11 | 49.2 |
| **198** ***(sp$^{+}$,rin, ls, u)*** | 83.6 ± 1.86 | 75.5 ± 2.05 | 77.2 ± 2.13 | 78.9 ± 2.69 | 21.3 |
| **204** ***(sp$^{\pm}$, ls, t, rin)*** | 80.2 ± 1.67 | 79.6 ± 1.54 | 75.7 ± 1.77 | 78.5 ± 1.73 | 21.5 |
| **205** ***(sp$^{+}$, ls, u)*** | 89.8 ± 1.78 | 90.9 ± 1.87 | 80.4 ± 1.41 | 89.7 ± 1.94 | 10.3 |
| **228** ***(sp$^{\pm}$, ls, u, ex)*** | 69.4 ± 1.82 | 72.4 ± 2.12 | 57.2 ± 1.28 | 66.3 ± 2.33 | 33.7 |
| **234** ***(sp$^{\pm}$, u ,nor, ls, br)*** | 55.9 ± 1.91 | 49.6 ± 1.67 | 45.4 ± 1.33 | 50.3 ± 1.87 | 49.7 |
| **237** ***(sp$^{\pm}$, rin, ls, br)*** | 73.5 ± 2.31 | 52.8 ± 2.17 | 36.3 ± 2.04 | 54.2 ± 2.05 | 45.8 |
| **241** ***(sp$^{+}$, ls, br, u)*** | 63.2 ± 1,34 | 65.7 ± 1.93 | 54.8 ± 1.74 | 61.2 ± 2.11 | 38.8 |
| **Average by tiers** | **79.2** | **77.0** | **70.1** | | |
| **$NSR_{05}$** | **3.71** | **3.14** | **3.96** | | |
| **Note:** 500 pollen grains were assessed for each line | | | | | |

### *3.3. Pollen Viability*

Differences between the lines were also established in pollen viability, which characterizes the pollen grain's fertilizing ability or zygotic potential. The pollen viability of the lines varied from very low (16.1%) to high (53.0%) (Table 3). At the same time, it should be noted that the study's results did not establish a direct connection between fertility and pollen viability.

For example, pollen from line 195, with very high pollen fertility (97.4%), had the lowest viability (16.1%), while line 196, with the lowest pollen fertility (43.4%), had 19.9% viability. Relatively high viability - 25.5%, 33.5%, 41.2 and 35.4% were detected in lines 197, 228, 237 and 241 with low fertility (50.8%, 66.3%, 54.2% and 61.2%, respectively) when germinating pollen on an artificial nutrient medium under in vitro conditions. In general, in the studied lines, pollen viability is 1.5 - 3 or more times lower than its fertility (Table 3).

From this, it follows that in flowers, there are significantly fewer lines of pollen grains capable of germinating and producing fertilization than fertile ones. No patterns were identified regarding the relationship between the indicators of these two characteristics.

Probably, the heterogeneity of lines in the composition of hereditary factors, including mutant genes, of which they are carriers, leads to the disruption of trophic relationships in the process of microsporogenesis, which is reflected in morphological heterogeneity and selective death of gametes. This, in turn, leads to the fact that gametes with the highest life potential survive and remain, which, when germinating, simultaneously form long pollen tubes.

### *3.4. Reaction of Pollen from Tomato Lines to High Temperature and Osmotic Stress*

The results of assessing the pollen of multi-marker tomato lines for resistance to high temperature and drought (osmotic factor) in terms of germination and the ability of pollen grains that sprouted under the influence of these stress factors to form tubes of sufficient length for fertilization showed their different responses to the action of these stressogens. Lines 178, 181, 195, 196, 197, 198, 201, 205, 237, and 241 had high indicators after high-temperature treatment of pollen for both traits (Table 3), which indicates their resistance to high temperature. The pollen of the remaining lines germinated worse under *in vitro* conditions after being treated at 45°C for 8 hours, but the germinated pollen grains formed long pollen tubes, which indicates a high zygotic potential and characterizes them as stable according to the "length of pollen tubes" trait. (Table 3). Germination on an artificial nutrient medium with a high concentration of sucrose of 38%, which simulates drought, has a strong inhibitory effect on pollen germination and the growth of pollen tubes. Against this background, only lines 181, 205, 237 and 241 stand out, the pollen of which germinates well, simultaneously forming long pollen tubes (Table 3).

Pollen from lines 110, 164, 178 and 234 germinates well against the background of the osmotic factor, but the germinated pollen grains cannot form pollen tubes of sufficient length for fertilization. Pollen from line 183 did not germinate. The remaining lines show different levels of resistance in terms of pollen germination and tube length. In most of the lines, the indicator of the trait "resistance along the length of pollen tubes" was very low and varied within the group from 10.1% to 28.0% (Table 3).

**Table 3. Indicators of traits characterizing the quality of pollen of multi-marker tomato lines and its resistance to stress factors**

| Lines | Fertility Pollen, % | Viability, % 25˚C (control) | Resistance, % | | | |
|---|---|---|---|---|---|---|
| | | | by Germination Pollen | | by Length Pollen Tubes | |
| | | | High Temperature | Drought | High Temperature | Drought |
| Л 110 | 83.7 ± 2.3 | 16.9 ± 1.0 | 26.0 ± 1.0 | 55.9 ± 1.6 | 46.9 ± 1.8 | 22.5 ± 1.1 |
| Л 164 | 96.1 ± 2.1 | 31.3 ± 1.2 | 18.9 ± 1.4 | 45.0 ± 1.7 | 60.0 ± 1.5 | 26.0 ± 1.6 |
| Л 178 | 90.2 ± 2.7 | 53.0 ± 1.2 | 80.4 ± 1.9 | 43.0 ± 1.1 | 99.1 ± 1.8 | 26.0 ± 1.2 |
| Л 181 | 92.1 ± 2.0 | 27.9 ± 1.1 | 43.1 ± 1.7 | 52.2 ± 1.5 | 73.0 ± 1.9 | 36.0 ± 1.0 |
| Л 183 | 85.5 ± 2.3 | 35.1 ± 1.6 | 24.0 ± 1.1 | 0,1 | 80.0 ± 2.0 | 0 |
| Л 188 | 91.4 ± 3.0 | 30.0 ± 1.9 | 36.3 ± 2.0 | 2.3 ± 0.4 | 70.1 ± 1.5 | 10.1 ± 0,2 |
| Л 195 | 97.4 ± 3.1 | 16.1 ± 0.4 | 52.0 ± 1.3 | 7.0 ± 0.5 | 73.0 ± 1.9 | 13.1 ± 0.6 |
| Л 196 | 43.4 ± 2.8 | 19.9 ± 1.0 | 48.8 ± 2.0 | 7.8 ± 0.3 | 89.8 ± 1.1 | 28.0 ± 0.7 |
| Л 197 | 52.5 ± 2.4 | 25.5 ± 1.8 | 83.0 ± 2.1 | 19.0 ± 0.9 | 70.7 ± 1.7 | 18.6 ± 0.9 |
| Л 198 | 78.9 ± 1.1 | 21.5 ± 0.8 | 78.0 ± 1.4 | 31.3 ± 1.7 | 86.8 ± 1.4 | 24.0 ± 0.9 |
| Л 201 | 86.8 ± 2.3 | 23.0 ± 0.6 | 50.9 ± 1.3 | 12.1 ± 0.6 | 90.0 ± 1.6 | 21.0 ± 1.0 |
| Л 204 | 78.5 ± 1.6 | 45.9 ± 1.7 | 24.7 ± 1.1 | 27.1 ± 2.0 | 79.8 ± 1.3 | 18.2 ± 1.1 |
| Л 205 | 89.7 ± 1.3 | 23.0 ± 1.7 | 57.4 ± 1.7 | 41.2 ± 2.0 | 61.3 ± 1.3 | 58.9 ± 1.6 |
| Л 228 | 66.3 ± 3.3 | 33.5 ± 1.3 | 32.0 ± 1.0 | 15.8 ± 1.1 | 96.9 ± 2.0 | 15.6 ± 0.4 |
| Л 234 | 50.3 ± 1.8 | 16.7 ± 0.7 | 18.0 ± 0.5 | 34.9 ± 0.9 | 42.0 ± 1.8 | 13.0 ± 1.0 |
| Л 237 | 54.2 ± 1.2 | 41.2 ± 1.3 | 48.8 ± 1.0 | 30.4 ± 1.5 | 68.4 ± 2.2 | 51.3 ± 1.4 |
| Л 241 | 61.2 ± 1.7 | 35.4 ± 0.7 | 52.6 ± 0.5 | 43.7 ± 1.1 | 4.8 ± 1.4 | 57.2 ± 0.9 |

Equally high resistance to both stress backgrounds for pollen germination and its ability to form long pollen tubes was detected in lines 205, 237 and 241 (Table 3). No pronounced differences were found between the lines carrying one *ls* gene or two *ls* and *br* genes in terms of resistance to high temperature and drought in pollen germination and the length of pollen tubes, which characterize the fertilizing ability of their male gametophyte.

### *3.5. Dispersion Factor Analysis of the Sources of Variability*

Dispersion Factor Analysis of the Sources of Variability in pollen resistance to abiotic stress factors revealed the share of the influence of genotype, stress factors and their interaction in the total variability of traits (Fig. 1). When individually analyzing the effect and interaction of factors on the nature of the manifestation of male gametophyte traits, it was revealed that in a number of lines (181, 183, 188, 193, 195, 201 and 241), the proportion of variability introduced by stress factors was high (from 47.2% to 58.9%), while in lines 110, 164, 178, 196, 198, 234 and 237 the share of the influence of stress factors was insignificant and varied from 7.5% to 26.3% (Fig. 1).

The nature of the manifestation of traits: fertility, viability, resistance to high temperature and drought for pollen germination and the ability of pollen grains germinated under the influence of stress factors to form pollen tubes of sufficient length for fertilization in these lines is determined by the genotype with the corresponding indicators of 70.0%, 69.6%, 74.6% 61.0%, 53.9%, 56.2% and 71.6%. From Figure 1, it can be seen that the share of variability introduced by the genotype and stress factors is individual for each line. Most of the multi-marker lines demonstrate a high genetically determined level of adaptability to the studied unfavorable environmental factors based on the characteristics of the male gametophyte.

### *3.6. Morphological Characteristics of Pollen Grains*

The effective use of these lines in the creation of heterotic hybrids requires the availability of information about the morphological characteristics of pollen grains, including ranking them by diameter depending on the genotype, the influence of the level of inflorescence initiation on the plant and the high-temperature factor. This is especially important when artificially interfering with the processes of pollination and fertilization, in particular during hybridization and industrial hybrid seed production. Differences were established in the average value of the trait "diameter of pollen grains", which varied from 20.4 µm to 29.6 µm, determined by the genotypic characteristics of the lines. Lines (196, 228, 234 and 241) with large pollen grains (28.3 µm, 29.6 µm, 28.3 µm and 25.9 µm, respectively) were identified.

The pollen of line 196 was uniform in the diameter of pollen grains, regardless of the level of inflorescence initiation. Pollen populations of lines 228, 234 and 241 are more heterogeneous due to the presence of pollen grains of different sizes, with deformations of varying degrees and impaired integrity, stuck together. The coefficient of variation (V, %) in the size of pollen grains from the lower to the upper inflorescences is 16.4%, 14.2 and 21.3%, respectively. In flowers of inflorescences at a higher position on the plant, there are more small pollen grains, but they practically do not respond to exposure to high temperatures. Whereas the treatment of pollen obtained from flowers of inflorescences of the lower and middle tiers with high temperatures significantly reduces their size (Table 4). At the same time, it should be noted that lines 204 and 205 were identified, which form small, uniform pollen grains regardless of the level of position of the inflorescences on the plant. The coefficient of variation in the diameter of pollen grains from the lower tier of the inflorescence position to the upper ones is very low and ranges from 3.7% to 5.4%. After high-temperature exposure, the size of pollen grains does not change regardless of the flowers of the inflorescences and at what level of position on the plant it was obtained (Table 4). At the same time, lines (197 and 198) were also identified, the pollen grains of which decrease in size as the level of inflorescence initiation on the main stem of the vegetative plant increases, with coefficients of variation of 19.3% and 20.8%. This pattern also persists when pollen is exposed to high temperatures (Table 4).

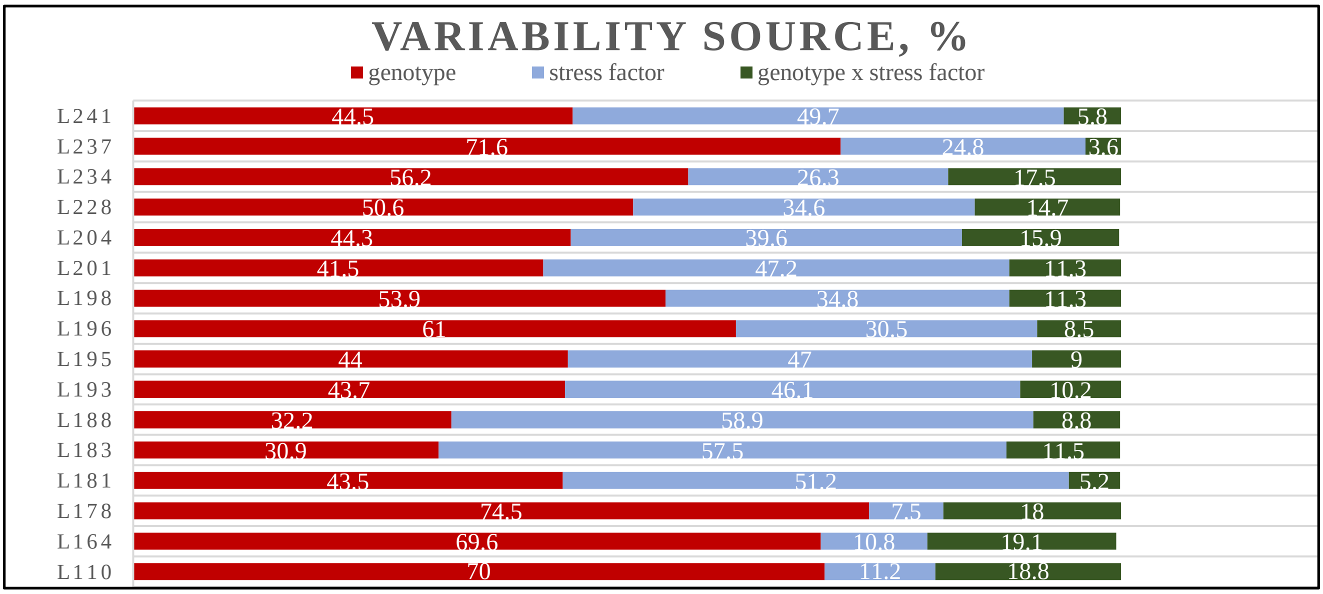


**Fig. 1 Factor analysis of sources of variability in pollen resistance to abiotic stresses in multi-marker lines**

A strictly defined pattern in the variability of the trait "pollen grain size" depending on the level of inflorescence position on the plant and the reaction of pollen lines to high temperature has not been established. The differences are determined by the genotypic characteristics of the lines. Some (1, 2, 10) form large grains on all plant inflorescences but react strongly to high temperatures, decreasing in size. Others (6, 7), on the contrary, having very small pollen, are characterized by a complete lack of response to high temperature. At the same time, lines (5 and 8) were also identified; the size of pollen grains decreases as the level of the inflorescence position increases; this sequence is maintained even after high-temperature action. This is especially pronounced in lines whose genome contains two mutant genes *ls* and *br*. It can be assumed that the main reason for the intralineal morphological variation in pollen quality, depending on the location of the inflorescence, is the different physiological states of plants in ontogenesis and the individual reaction of genotypes to these changes. Based on this, when carrying out hybridization, the breeder must take into account the genotypic characteristics of the maternal and paternal forms, their reaction to high-temperature conditions, and the inflorescences' location on the plant.

**Table 4. Sizes of pollen grains in tomato lines before and after heat treatment relative to the tier of inflorescence location on the main stem of the plant**

| Lines | Diameter of pollen grains of freshly collected pollen, µm | | | Diameter of pollen grains after heat treatment, µm | | |
|---|---|---|---|---|---|---|
| | Inflorescences | | | Inflorescences | | |
| | 1-2 | 3-4 | 5-6 | 1-2 | 3-4 | 5-6 |
| 1. L 166 | 28,9 ± 0,11 | 30,0 ± 0,14 | 30,0 ± 0,12 | 22,0 ± 0,20*** | 29,0 ± 0,16* | 27,0 ± 0,11*** |
| 2. L 167 | 30,0 ± 0,18 | 32,9 ± 0,16 | 28,0 ± 0,14 | 30,0 ± 0,19 | 27,6 ± 0,19*** | 27,6 ± 0,17 |
| 3. L 168 | 26,0 ± 0,20 | 27,0 ± 0,16 | 27,4 ± 0,18 | 26,0 ± 0,11 | 25,0 ± 0,18*** | 27,0 ± 0,14 |
| 4. L 169 | 25,0 ± 0,16 | 26,3 ± 0,21 | 22,0 ± 0,20 | 23,0 ± 0,17** | 23,0 ± 0,15*** | 22,0 ± 0,15 |
| 5. L 170 | 29,0 ± 0,15 | 28,0 ± 0,11 | 27,0 ± 0,17 | 25,0 ± 0,14*** | 24,0 ± 0,19*** | 22,0 ± 0,12*** |
| 6. L 171 | 20,0 ± 0,10 | 21,0 ± 0,13 | 20,0 ± 0,10 | 21,0 ± 0,10* | 20,0 ± 0,13* | 21,1 ± 0,17** |
| 7. L 172 | 23,0 ± 0,15 | 23,0 ± 0,14 | 24,0 ± 0,13 | 23,2 ± 0,18 | 22,6 ± 0,21 | 24,0 ± 0,13 |
| 8. L 173 | 33,1 ± 0,21 | 26,0 ± 0,15 | 23,0 ± 0,11 | 29,0 ± 0,18*** | 25,6 ± 0,19* | 22,4 ± 0,12* |
| 9. L 174 | 26,1 ± 0,17 | 21,0 ± 0,11 | 25,0 ± 0,15 | 26,1 ± 0,15 | 20,8 ± 0,23 | 24,9 ± 0,11 |
| 10. L175 | 28,0 ± 0,10 | 29,0 ± 0,18 | 29,0 ± 0,21 | 19,6 ± 0,10*** | 20,0 ± 0,13*** | 23,0 ± 0,09*** |
| The differences are significant: * - at the 0.05% level, ** - 0.01% and *** 0.001% | | | | | | |

These and the above characteristics show that new multimarker lines are characterized by a complex of complementary morphobiological and other economically valuable traits and represent genetically valuable material for subsequent use in breeding programs, including in heterotic selection as paternal components (pollinators).

## 4. Discussion

The identified wide range of trait variability made it possible to fairly fully reflect the hereditary genetically determined potential of individual variability in fertility and pollen viability of freshly collected pollen, as well as the response of pollen of multi-marker tomato lines to the action of abiotic stress factors - high temperature and drought. At the same time, the influence of the level of inflorescence initiation on the main stem of a vegetative plant in multi-marker lines on the nature of the variability of the studied characteristics of the male gametophyte (pollen viability; length of pollen tubes, resistance to pollen germination; stability to the length of pollen tubes; morphobiological characteristics of pollen grains), including depending on the genotype and the action of the high-temperature stress factor. It has been established that each line is characterized by genetically determined individual variability in the characteristics of the male gametophyte, the magnitude of which simultaneously depends on the age characteristics of the plant and environmental factors, but at the same time, different characteristics of one genotype change to an unequal extent, remaining within the limits of the reaction norm of each specific genotype on the effect of one or another factor. This can be valuable and widely used in heterotic breeding when selecting at the initial stages of lines as paternal components (pollinators). This also indicates the feasibility and effectiveness of using artificially created stress backgrounds for the assessment, identification and selection of resistant genotypes based on the response of their pollen to the action of various stress factors, thereby promoting the intensification of selection for the creation of initial parental forms, hybridization and industrial hybrid seed production. Consequently, pollen traits characterizing its quality can serve as an effective criterion for selecting breeding-valuable material for use in conventional and heterotic breeding. This non-standard approach to research, taking into account the complex characteristics of the male gametophyte (pollen), allows, at the early stages of the selection process in the laboratory under artificially created stress backgrounds, the evaluation of extensive material and thereby intensifies the process of isolating genotypes with a stable manifestation of traits for their subsequent use in selection tomato as a starting material.

## 5. Conclusion

There is no direct connection between fertility and pollen viability, which characterizes its zygotic potential. Strongly pronounced genotypic differences were revealed between multi-marker lines in the response of their pollen to high temperature and osmotic stress. According to the resistance of pollen lines to high temperatures, the values of the trait varied from 18.0% (L234) to 83.1% (L193). The indicators for pollen germination of lines against the background of the osmotic factor were also contrasting - from 0.1% (L183) to 55.9 (L110). This shows the differential response of pollen of different genotypes to the action of stressful abiotic factors, allowing us to identify the most resistant ones. A definite pattern in the variability of the trait "diameter of pollen grains" depending on the level of position of the inflorescence on the plant or the reaction of pollen to exposure to high temperature has not been established. The nature and degree of variability of the trait are determined by the genotype, and the variability of its indicator is higher in lines whose genome contains two mutant genes ls and br. Dispersion factor analysis of the sources of variability in pollen resistance to stress abiotic factors revealed the share of the influence of genotype, stress factors and their interaction in the overall variability of traits, which indicates the effectiveness and feasibility of using different traits of the male gametophyte as a criterion for assessing, identifying and selecting stress-resistant genotypes factors for subsequent use in breeding.

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## References

[1] F. Alanga et al., "Getting Ready with the Priming: Innovative Weapons against Biotic and Abiotic Crop Enemies in a Global Changing Scenario," *Priming-Mediated Stress and Cross-Srtess Tolerance in Crop Plants*, pp. 35-56, 2020. [CrossRef] [Google Scholar] [Publisher Link]

[2] Beny Aloni et al., "The Effect of High Temperature and High Atmospheric $CO_2$ on Carbohydrate Changes in Bell Pepper (*Capsicum Annuum*) Pollen in Relation to its Germination," *Physiologia Plantarum*, vol. 112, no. 4, pp. 505-512, 2001. [CrossRef] [Google Scholar] [Publisher Link]

[3] Klaus Apel, and Heribert Hirt, "Reactive Oxygen Species: Metabolism, Oxidative Stress, and Signal Transduction," *Annual Review of Plant Biology*, vol. 55, pp. 373-399, 2004. [CrossRef] [Google Scholar] [Publisher Link]

[4] T.B. Batygina et al., *From Microspores to Varieties*. Moscow, Sprint-Nauka, 2010.

[5] Stephen Blackmore et al., "Pollen Development in Flowing Plants," *New Phytologist*, vol. 174, no. 3, pp. 483-498, 2007. [CrossRef] [Google Scholar] [Publisher Link]

[6] Damtew Abewoy Fentik, "Review on Genetics and Breeding of Tomato (Lycopersicon Esculentum Mill)," *Advanced in Crop Science and Technology*, vol. 5, no. 5, 2017. [CrossRef] [Google Scholar] [Publisher Link]

[7] I.I. Golubinsky, *Biology of Pollen Germination*, Kyiv: Sprint-Naukova Dumka, 1971. [Publisher Link]

[8] Sun-Yuan Hsu, Yih-Ching Huang, and P.A. Peterson, "Development Pattern of Microspores in Zea Mays L. The Maturation of Upper and Lower Florets of Spikelets among an Assortment of Genotypes," *Maydica*, vol. 33, no. 2, pp. 77-98, 1988. [Google Scholar] [Publisher Link]

[9] R. Izmailow, "The Effect of Soil from Polluted Sites on Reproductive Success in Ranunculus Repens (Ranunculaceae)," *Polish Botanical Studies*, vol. 15, pp. 5-10, 2002. [Google Scholar]

[10] Christian Jung, and Andreas E. Müller, "Flowering Time Control and Application in Plant Breeding," *Trends Plant Science*, vol. 14, no. 10, pp. 563-573, 2009. [CrossRef] [Google Scholar] [Publisher Link]

[11] A.A. Zhuchenko, *Adaptive Crop Production (Ecological and Genetic Foundations) Theory and Practice*, Moscow, Sprint-Agrorus, vol. 2, pp. 647-651, 2009. [Google Scholar]

[12] Alexander Alexandrovich Zhuchenko, "Mobilization of World Resources of Flowering Plants based on Creation of Systematized Genetic Collections," *Vegetables of Russia*, no. 4, pp. 4-13, 2012. [CrossRef] [Publisher Link]

[13] Manjit S. Kang, "Breeding: Genotype-by-Environment Interaction," *Encyclopedia of Plant and Crop Science*, New York: Marcel-Dekker, pp. 218-221, 2004. [Google Scholar] [Publisher Link]

[14] V.G. Kakani et al., "Response of in Vitro Pollen Germination and Pollen Tube Growth of Groundnut (Arachis Hypogaea L.) Genotypes to Temperature," *Plant, Cell and Environment*, vol. 25, no. 12, pp. 1651-1661, 2002. [CrossRef] [Google Scholar] [Publisher Link]

[15] A.V. Kilchevsky, and L.V. Khotyleva, *Genetic Bases of Plant Breeding*, Minsk: Belarus Science, vol. 1, 2008.

[16] O.E. Klimenco, and N.I. Klimenco, "Effects of Artificial Acid Rain on Stone Fruit Pollen Vitality and Performance Fruiting," *Journal of Horticulture*, vol. 17, pp. 186-191, 2005.

[17] A.N. Kravceno, V.A. Lyakh, and L.G. Toderash, *Methods of Gamete and Zygote Selection of Tomatoes*, Kishinev, 1988.

[18] V.A. Lyakh, M.G. Kalinova, and A.I. Soroka, "Microgametophyte Selection for Resistance to Low Temperatures in Spring Rapeseed," *Cytology and Genetics*, no. 3, pp. 71-76, 1997.

[19] V.A. Lyakh, and A.I. Soroka, "Pollen Selection as a Way to Intensify the Selection of Oilseeds", *Scientific and Technical Bulletin of the Institute of Olive Cultures of the National Academy of Sciences of Ukraine*, no. 20, pp. 72-80, 2014.

[20] B. Maisonneuve, N.G. Hogenboom, and A.P. Den Nijs, "Pollen Selection in Breeding Tomato (Lycopersicon Esculentum Mill.) for Adaption to Low Temperature," *Euphytica*, vol. 35, no 3, pp. 983-992, 1986. [Google Scholar]

[21] G.P. Mascarenhas, and Mitcel Altshuler, "The Response of Pollen to High-Temperatures and its Potential Applications," *Pollen: Biology and Implications for Plant Breeding*, pp. 3-8, 1982. [Google Scholar]

[22] Matamoro-Vidal et al., "Evolutionary Stasis in Euphorbiaceae Pollen: Selection and Constraints," *Journal of Evolutionary Biology*, vol. 25, no. 6, pp. 1077-1096, 2012. [CrossRef] [Google Scholar] [Publisher Link]

[23] D.L. Mulcahy et al., "Pollen Selection for Stress Tolerance or the Advantage of Selecting Before Pollination," *Sexual Reproduction in Higher Plants*, pp. 43-50, 1988. [CrossRef] [Google Scholar] [Publisher Link]

[24] E. Ottaviano, and M.Sari-Gorla, "Gametophytic and Sporophytic Selection," *Plant Breeding Principles and Prospects*, pp. 333-352, 1993.

[25] Z.P. Pausheva, *Workshop on Plant Cytology*, Moscow. Sprint-Nauka, 1988. [Publisher Link]

[26] B.S. Patil, R.L. Ravicumar, and P.M. Salimath, "Effect of Pollen Selection for Moisture Stress Tolerance on Progeny Performance in Sorghum," *Journal of Food, Agriculture & Environment*, vol. 4, no. 1, pp. 201-204, 2006. [Google Scholar] [Publisher Link]

[27] P.L. Pfahler, "Comparative Effectiveness of Pollen Genotype Selection in Higher Plants," *Pollen: Biology and Implications for Plant Breeding*, pp. 361-366, 1982. [Google Scholar]

[28] Etan Pressman, Mary M. Peet, and D. Mason Pharr, "The Effect of Heat Stress on Tomato Pollen Characteristics is Associated with Changes in Carbohydrate Concentration in the Developing Anters," *Annals of Botany*, vol. 90, no. 5, pp. 631-636, 2002. [CrossRef] [Google Scholar] [Publisher Link]

[29] R.D. Reyes, and M.G. Simpson, "Preliminary Investigation of Pollen Development of Philydrum *Lanuginosum*," *Microscopy Research and Technique*, vol. 36, no. 4, pp. 350-352, 2007. [Google Scholar]

[30] Ashutosh Singh, Rajen Chowdhury, and Ratan Das, "Gametophytic Selection: A Simple Technique for Thermo Tolerance Genotypes Identification in Maize," *International Journal of Current Microbiology and Applied Sciences*, vol. 6, no. 8, pp. 1649-1655, 2017. [Google Scholar] [Publisher Link]

[31] M.P. Solntseva, and K.P. Glazunova, "Influence of Industrial and Transport Pollution of the Environment on the Reproduction of Seed Plants," *General Biology*, vol. 71, no. 2, pp. 163-175, 2010. [Google Scholar]

[32] Nobuhiro Suzuki, and Ron Mittler, "Reactive Oxygen Species and Temperature Stresses: A Delicate Balance between Signaling and Destruction," *Physiologia Plantarum*, no. 126, pp. 45-51, 2006. [CrossRef] [Google Scholar] [Publisher Link]

[33] G.V. Udovenco, "Plant Resistance to Adverse Environmental Conditions and Selection," *VIR Bulletin*, vol. 86, pp. 3-4, 2011.

[34] Yeug-Kyung Chang et al., "Effect of Temperature on Gametophytic Selection in a Phalaenopsis $F_1$ Population," *Euphytica*, vol. 171, no. 2, pp. 251-261, 2010. [CrossRef] [Google Scholar] [Publisher Link]