TWV/31/6
ORIGINAL: English
DATE: October 24, 1997

UPOV

INTERNATIONAL UNION FOR THE PROTECTION OF NEW VARIETIES OF PLANTS

GENEVA

TECHNICAL WORKING PARTY FOR VEGETABLES

Thirty-First Session
Valencia, Spain, November 24 to 28, 1997

POPULATION STANDARDS IN HYBRIDS OF OUTBREEDING SPECIES

Document prepared by experts from the Netherlands

1. During the TWV 30 meeting in Brno (Czech Republic) differences in opinion occurred about the application of homogeneity standards in (especially) hybrids of outbreeding species.

2. Document TG/1/2 gives the basic principles for homogeneity testing, especially paragraphs 27 to and including 34 deal with this subject. Paragraph 28 deals with the standards for vegetatively propagated and truly self-pollinated varieties; 30 to 32 with the cross-pollinated and synthetic varieties and paragraphs 33 and 34 with hybrid varieties.

3. Document TWC/11/16 (Proposal for the replacement of paragraph 28 of TG/1/2 and revision of TC/XXV/8) gives an extensive proposal for new decision standards replacing paragraph 28 of TG/1/2. This proposal has basically been accepted by the Technical Committee but needed a better understandable redaction. So far I did not see a final proposal or document (see paragraph 29 of the report TC/33/11). Nevertheless this document is especially meant for vegetatively and truly self-pollinated crops.

4. During our last meetings (since Denmark) we included proposals for population standards in our guidelines proposals <u>also for hybrids</u>. In Brno when we discussed this matter again for hybrids of some outbreeding species (e.g. beetroot, leaf chicory, spinach and the Alliums) I realized that we propose to apply these principles designed for genetically homogeneous species/varieties to hybrid varieties which do not have such a genetic homogeneous constitution. This paper is meant to rediscuss this matter more thoroughly.

<u>Way of propagation and Genetic Homogeneity</u>

5. Document TG/1/2 splits the recommendations for homogeneity standards according to the genetic constitution of the species/varieties involved. From a genetic point of view the following splitting has been used:

(a) *vegetatively propagated and truly self-pollinated species*: all plants of the variety can and should have the identical genetic constitution. Deviations may occur because of mutations and in self-pollinated species for unselected characteristics if the variety (theoretically) is bred for less than 11 generations. These true-breeding varieties may be used as parent lines for hybrid seed production (e.g. tomato).

(b) *mainly self-pollinated species:* a number of plants contain certain genes resulting from cross-pollination and/or introgression. Genetic homogeneity may vary slightly. Standards have to be defined case by case and based on experience.

Parent lines for hybrids in these species will mainly be treated as truly self-pollinated; F1-hybrids will be fairly homogeneous genetically (e.g. sweet pepper, cucumber).

(c) *cross-pollinated and synthetic species*: depending on the breeding background and the degree of selection and/or inbreeding the genetic constitution may vary quite widely. Standards need to be relative standards, which means “in comparison to” other varieties of the same type.

(d) *hybrids of truly self-pollinated species*:

– *single crosses*: these hybrids will be very homogeneous genetically, depending on the mechanism of hybridization and its failures (see (a)).

– *other categories of hybrids*: the genetic constitution of the hybrids will very much depend on the formula of the hybrid and/or the failures in the mechanism of hybridization. A clear prediction cannot be given. From experience, standards for homogeneity might be derived.

(e) *hybrids of naturally cross-pollinated species*: (see also Annex 1)

Because breeding techniques have offered a wide range of solutions to overcome the problems connected with the production of F1-hybrid varieties, especially in outbreeding species, no unequivocal prediction of the genetic homogeneity is feasible. Matters become even more complicated because of incomplete functioning of the mechanisms used in hybrid seed production. A number of special situations and the consequences for the genetic homogeneity in inbred lines and F1-hybrids are listed and discussed:

(i) Single cross hybrids derived from *doubled haploid parent lines* will generally be very homogeneous (e.g. asparagus).

(ii) If *no or hardly any inbreeding depression* occurs in the parent lines, these can be bred for at least 7 to 10 generations and become fairly homogeneous. Single cross hybrids produced using such parent lines will be fairly homogeneous genetically. Double crosses will be less homogeneous.

Other hybrid formulas and imperfect functioning of the hybridization mechanism may lead to unpredictable genetic in-homogeneity. Standards for homogeneity will have to be derived from experience and the hybrid formula (if known).

(iii) In crops where *inbreeding depression is a serious drawback* for economic production of hybrid seed the genetic constitution of the parent lines will only be homogeneous for a number of important characteristics. The unselected genes tend to reach a Hardy-Weinberg equilibrium, but could always be the source of unexpected heterogeneity in F1 hybrids. In general hybrids will be genetically heterogeneous to some degree. In case of a tetraploid species (e.g. leek) the situation is even worse.

Crops like carrot, onion, leaf chicory, cabbage, brussels sprouts and leek do not allow an extensive inbreeding program: After three to four generations of inbreeding the economic threshold has been reached. Therefore some sophisticated hybrid formula have been developed to overcome the production problems e.g. double crosses using isogenic lines.

(iv) The *mechanism used for the production* of the hybrid seed also has consequences for the degree of homogeneity. Sporophytic incompatibility, cytoplasmic and genetic male sterility and certation may not always function completely (e.g. because of non-simultaneous flowering or incomplete functioning of male sterility). This may result in ‘sibs’ or inbred plants, which will not always be recognizable as such, e.g. in carrot and leek. If ‘sibs’ can be recognized, special tolerances for them may be applied.

(v) Another source of observed variation (phenotype) are environmental influences, such as soil conditions, drilling depth, humidity conditions. Especially in root and bulbous crops growing (partly) in the soil this occurs frequently. In case of recommendation of clearly defined 'population standards' for homogeneity in these crops special standards are needed for these characteristics.

(vi) The hybrid formula (type of hybrid) has a strong influence on the genetic homogeneity of the hybrid variety:

– single cross: genetic variation will be present for those genes/alleles which are not homogeneously present in the parent lines,

– threeway crosses: the genetic constitution of the parent lines may result in a wider variation of phenotypes. Any prediction on homogeneity is impossible. Standards for decision need to be derived from existing varieties of the same type within the species, like for cross-pollinated species,

– double crosses: the genetic constitution of the final hybrid is less predictable than for threeway cross hybrids. Again the standards for decision on homogeneity need to be derived from the existing varieties of the same type.

– Combination of more than 4 lines hardly occurs in hybrid breeding, but if so the genetic constitution tends to become slightly restricted in comparison to synthetics and OP's.

Hybrid varieties of naturally cross-pollinating species will generally show variation in the expression of their characteristics, because their genetic constitution will hardly be identical within the variety. This variation will be less than for standard outbreeding OP's.

Population Standards

6. A 'population standard' is a characteristic of a population or in our situation a variety, which may even vary from one morphological characteristic to the other. On the other hand the genetic constitution of F1-hybrids in outbreeding species, although less variable than in the OP ancestors, can hardly be predicted.

7. Application of uniform 'population standards' for decisions on homogeneity in hybrids in naturally outbreeding species goes far beyond the principles of document TG/1/2. It supports the idea of high accuracy in testing but it does not take into account all the possible sources of genetic variation that occur in the material offered for testing. An approach according to that recommended for cross-pollinated species would be more feasible. As "the standard" the uniformity of other varieties with the same formula should be taken into account. If such a standard is not available, because it is the first variety with that hybrid formula, the already existing varieties could be the "measuring stick." In most cases such new hybrids will show a higher degree of uniformity than the existing varieties. Hybrids in out-breeding species tend to be more homogeneous than the open pollinated ancestors.

8. Moreover we should be aware that a number of characteristics in some crops are more strongly influenced by environmental conditions than others. The relative standard corrects for this, but a (low) population standard (5 instead of 1 or 2%) for all characteristics of such a variety would not be very adequate.

Conclusions

9. The principles of document TWC/11/16, which are especially meant for genetically homogeneous varieties should not be applied for hybrids in naturally cross-pollinating species especially if inbreeding depression plays an important role in the parent lines.

10. In case the "population standard" might become a general recommendation for all types of hybrids we should be aware of the consequences of this.

[Annex follows]

ANNEX

REMARKS TO THE DRAFT REPORT (TWV/30/21 PROV.)
DATED AUGUST 19, 1996

General:

In relation to document TWC/14/11 (referred to in TWV/30/21 Prov., par 23) I have clear reservations to the application of the decision standards of TWC/11/16 to F1-hybrids of naturally out-pollinated species, especially if only a few generations of inbreeding can be applied to prevent lack of vigor (e.g.: Red beet and Leaf chicory).

In our opinion F1-hybrids of Brassica's, Carrots, Onions, Leaf chicory and some other species do not fit to the model of the maize hybrids, because inbreeding depression is very strong within most of these species, although there are individual differences between inbred lines. As a result of only two to four generations of inbreeding the genetic structure of these inbreeds, and consequently of the hybrids, is still rather heterogeneous. This may result in hybrids that are much more homogeneous than the former population varieties, but still do not fit to the standards developed for self and vegetatively propagated species (for which document TWC/11/16 has been settled).

Explanation**:**

– In carrots, onions and some Brassica's the cytoplasmic male sterility principle is used to produce hybrids:

('A' * 'B') * 'C',

where 'A' is the male sterile parent, which is reproduced through pollination by 'B', the maintainer line; in a second cycle the hybrid is produced. In general three tot four inbred generations are applied for the inbred lines.

– In chicory so far the principle of certation (competition between own and foreign pollen) is used for the production of hybrids. This system is not always very reliable. "Inbred lines" can in general only be produced by about 3 generations of selfing, while the species is in fact rather heterogeneous.

– In most Brassica hybrids the system of sporophytic self-incompatibility is applied for the production hybrids, either single cross, threeway cross or double cross by using 'isogenic pairs'. In most Brassica's three to five inbred generations are applied for inbred lines.

Because of the low number of inbred generations in the parental material we can not expect the high standards of uniformity as in hybrids of naturally self-pollinated species like tomato. Moreover a number of essential characteristics are moderately to strongly influenced by environmental factors, e.g. root shape in beetroot, bulb shape characteristics in onions. For that reason I cannot understand that for hybrids population standards of 3% could be introduced in the guidelines for beetroot and large-leafed chicory. In our opinion only relative standards of similar hybrid varieties can be applied similar to open pollinated varieties.

[End of Annex and of document]