Plant Archives Vol. 26, Supplement 2, 2026 pp. 1632-1637 e-ISSN:2581-6063 (online), ISSN:0972-5210

Plant Archives

Journal homepage: http://www.plantarchives.org
DOI Url : https://doi.org/10.51470/PLANTARCHIVES.2026.v26.supplement-2.198

MULTILINE VARIETY: PRINCIPLE AND BREEDING METHODS

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(Date of Receiving : 22-03-2026; Date of Revision : 25-04-2026; Date of Acceptance : 30-05-2026)

ABSTRACT

Multiline variety is based on principle of diversification, ecology, compatibility of among component lines and population buffering. Intra-varietal diversification control disease by decreasing the rate of infection and limiting inoculum development/disease spread. It is a sustainable method which can overcome reliance on harmful chemicals to combat disease infection and consequently protect environment. Most importantly they also ensure yield stability. Disease resistance in plants are broadly classified under two groups: vertical resistance and horizontal resistance. Vertical resistance is effective against one specific race of a pathogen and hence not stable. Horizontal resistance is more durable however, breeding for horizontal resistance in a plant breeding program has its own complications. In such situation a synthetic horizontal resistance which is cumulative effect of *n* number of vertical resistance present in *n* number of component lines of a multiline can be one sustainable and durable solution for pathogen with evolving physiological races like rust. Multiline phenotypically similar and genotypically dissimilar and consist of such lines that can be reconstituted.
Keywords **:** Multiline, crop diversification, vertical resistance, horizontal resistance

Introduction

Plants have to defend itself against plethora of pathogens; and genotypes mixture in form of cultivar/variety mixture and multiline. Manifestation of diversification in form of cultivar/variety mixture and multiline can be one sustainable solution; albeit not in all cases. Diversification in any form possess considerable credibility in disease management. Multiline can be defined as mixture of several near isogenic lines that carry resistant gene for respective physiological races of a pathogen (Browning and Frey 1981, Reddy 2013). Jensen in 1952 and Borlaug in 1959, suggested application of multiline variety in oats and wheat respectively. Multiline cultivars carry both vertical and horizontal resistance (Browning and Frey 1981). A multiline variety is a mixture of compatible lines and acquire broader protection against disease (Jensen, 1952) and Nearly six backcrosses of a recurrent parent can give such lines which inherit 97% of RP genes and one added gene against the pathogen (Briggs and Allard, 1954). "Cultivar mixtures" is another concept used under principle of diversification against different races of a pathogen. It can be defined as mixture of component lines that are different from each other yet have similar agronomic traits hence can be grown together (Wolff, 1988). Cultivar mixtures has advantage over multiline that it does not require intensive breeding program (Mundt, 2002). In backdrop of a crop performance, stability concerns with the performance of plant population in adverse growth conditions. In simple words, a stable population will give considerable yield in adverse abiotic/biotic stress conditions also. Stability in plant performance is vital in context of multilines. A heterozygous and heterogenous populations exhibit more stability than a

homozygous or homogenous population (Acquaah 2016).

Properties of a multiline variety:

i. It has intra-varietal diversity (Jensen, 1952).
ii. Consist of several component lines: viz., isogenic lines (Browning and Frey, 1969), near isogenic lines (Browning and Frey, 1981), purelines (Jensen, 1952).
iii. Shows genotypic heterogeneity and phenotypic uniformity (Acquaah 2016).
iv. Longer varietal life (Jensen, 1952).
v. Multiline yield should be equivalent to average yield of all component lines of a multiline (Jensen, 1952).
vi. Efficient protection against disease (Jensen, 1952).
vii. Wide adaptation in different environment (Jensen, 1952).
viii. Acquire heterogenic horizontal resistance (Browning and Frey, 1969) synthetic horizontal resistance.
ix. Dynamic in nature (Wilson *et al*., 2001)

Principles of development of a multiline variety:

Synthetic horizontal resistance: A multiline cultivar contains several components lines each one carrying vertical resistance against a known physiological race of a pathogen. Thus, on the whole a multiline variety, possess horizontal resistance against a disease (Browning and Frey, 1969). Multiline variety with synthetic horizontal resistance were suggested in oats and wheat by Jensen (1952) and Borlaug & Gilber (1953) respectively.

Diversification is another principle which defines a multiline variety. When a mixture of plants genotypes is grown which shows different reaction to pathogen can efficiently reduce disease intensity. A multiline follows intra-varietal crop diversification. However, diversification leads to non-uniformity and consequently possess seed production constraints hence the diversification of components lines of a multiline should be precisely determined and controlled (Wolf, 1985).

Population buffering: The component lines of a multiline are genotypically diverse against the different races of a pathogen and consequently provide stability in yield even after disease infestation (Allard and Bradshaw, 1964; Marshall and Brown, 1973).

Appropriate proportion and compatibility of component lines: Appropriate proportion and compatibility of component lines influences the efficiency of a multiline. A randomly chosen component lines of a multiline may not give desirable results, therefore the components lines should be relevant and functional against the pathogen. (Mundt, 2002).

Stability: In backdrop of a crop performance, stability concerns with the performance of plant population in adverse growth conditions. In simple words, a stable population will give considerable yield in adverse abiotic/biotic stress conditions also. Stability in plant performance is vital in context of multilines. A heterozygous and heterogenous populations exhibit more stability than a homozygous or homogenous population (Acquaah 2016).

Methods of development of multiline variety:

Multiline variety can be developed either by selecting several purleines with similar phenotypes together and possess resistant genes against pathogen or by following a crossing program.

Procedure of developing a multiline variety following backcrossing breeding method:

In general, a multiline variety following crossing can be developed in four steps: 1) screening and collection of genotypes resistant to different known physiological races of a pathogen 2) development of lines each resistant to different physiological races of a pathogen; 3) evaluation of component lines for resistance gene and 4) equal mixing of different component lines to constitute a multiline. Development of resistant lines in aforementioned method can be done in two ways: i) carry out 5-6 backcrossing in the backcross program for each donor resistant lines separately with one recurrent parent; ii) carry out backcross for 2-3 times separately for each resistant donor parents with the chosen RP followed by pedigree method to obtain resistant lines at the end. A multiline variety can be also developed using single cross or complex crosses (Singh, 2015).

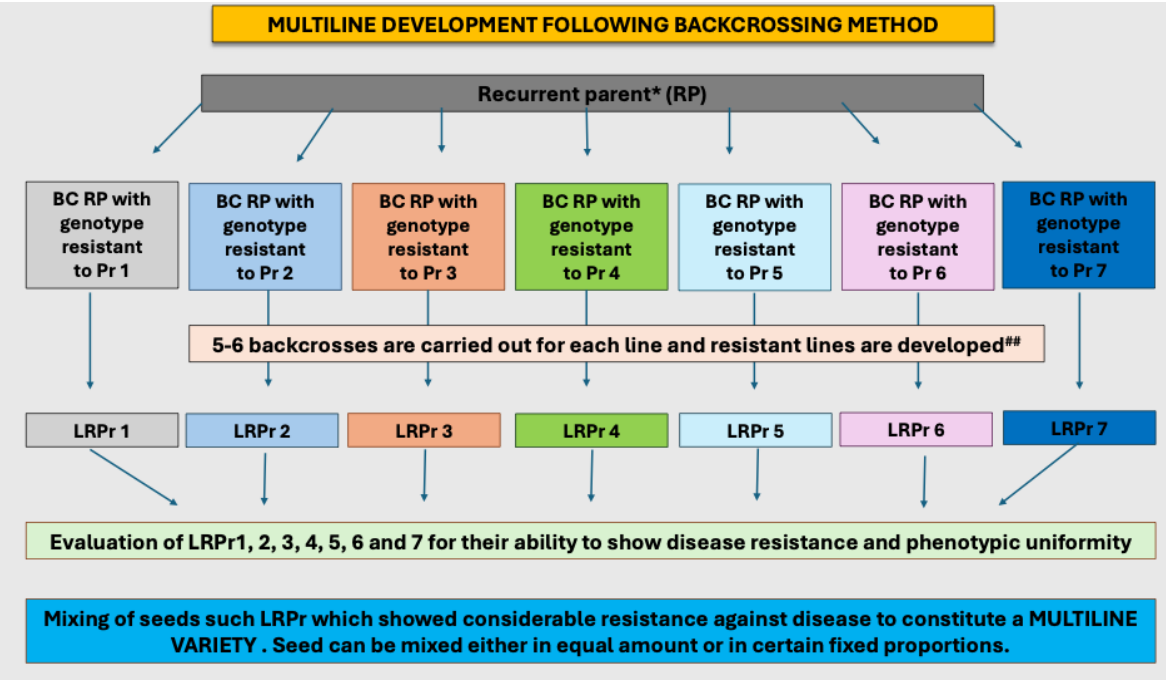

Fig 1. Steps of development of a multiline following a crossing program

a) Selection of a variety as recurrent parent (RP) **b)** screening of genotypes for disease resistance of different physiological races of a pathogen **c)** 5-6 backcrossing of RP separately with each of selected resistant lines against pathological races of a pathogen[##] **d)** screening of developed lines for resistance gene **e)** mixing of seeds of developed lines in equal amount.

*The Recurrent parent (RP) can be a *cultivated variety/pureline/high yield variety*

[##]Rather than backcrossing RP 5-6 times; after 2-3 backcrossing the lines developed can be handled via pedigree method of breeding to develop constituent lines of a multiline.

Physiological race-Pr; Backcross- BC; Line resistant to Physiological race- LRPr

Procedure of developing a multiline variety by mixing several purelines (Fig. 2)

Multiline can be also produced by incorporating several purelines that show uniformity in essential agronomic traits viz., height, maturity etc. and resistance to disease (Jensen, 1952). The component lines in such case should contain desirable genes and phenotypic uniformity (Jensen, 1952).

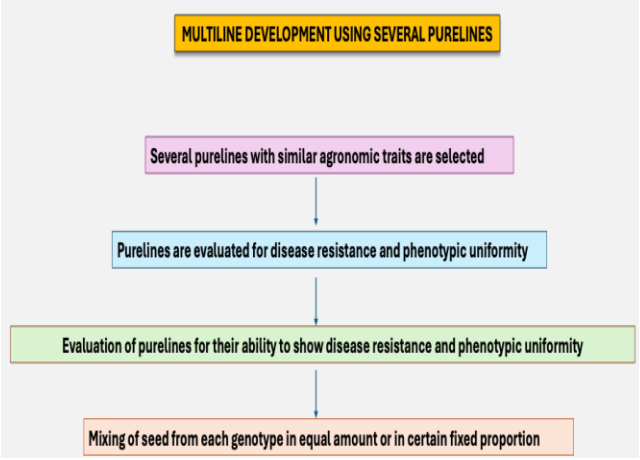

Fig. 2: Steps of development of a multiline using several purelines:

a) Several purelines are selected
b) Selected purelines are tested for disease resistance and phenotypic uniformity for crucial agronomic traits viz., plant height maturity etc.
c) Purelines showing disease resistance and phenotypic uniformity are selected
d) Seed from each selected purelines are either mixed in equal amount or fixed proportion to constitute a multiline variety

The final and important step in the development of a multiline is evaluation of its component lines. The component lines are tested on parameters like, yield, other agronomic traits and disease resistance to known physiological races of a pathogen. Evaluation of component lines are important because lines may can have neutral/positive/negative effect of each other (Singh, 2015). Each components lines should possess considerable amount of resistance against pathogen.

Number of component lines depends on the ultimate objective behind development of a multiline. For example, if durability of resistance is required then, lines should range between 15-20. However, if obligation is for lesser level of disease then accordingly the number of lines can be reduced. Overtime the resistant component lines of a multiline are liable to become susceptible and such lines are required to replace by resistant lines. Hence a plant breeder continuously searches for resistant genes to develop new resistant isogenic lines. Consequently, development of a multiline is a constant process.

Desirable qualities of component lines of a multiline

i. Should possess vertical resistance against different races of a pathogen
ii. Component lines should possess resistance against other diseases too.
iii. Resistance should last for good period of time.
iv. Should show phenotypic uniformity for crucial agronomic traits viz., plant height, maturity etc.
v. On the whole, all component lines together should carry yield advantage over a variety grown independently.

Advantages and Disadvantages of a multiline variety

Advantages of Multiline variety:

i. **Uniform population**: All the component lines are identical to recurrent parent on agronomic traits.
ii. **Loss in yield can be minimized**: As the population is heterogenous for a given pathogen different physiological races, hence even in worst case, substantial yield can be achieved. It stabilizes farm productivity.
iii. **Possess both VR and HR:** Since component lines carries VR and collectively a multiline show HR hence disease resistance of multiline is more durable.
iv. **Effect of pathogen on multiline:** At a time even if one or few races become susceptible therefore only small population of plants will be infected and hence damage is minimized. Risk of development of uniform pathogen population at large scale can be prevented. It stabilizes race composition of a pathogen.

Disadvantage of a multiline variety:

i. **Reconstitution of components lines of a multiline**: Components lines in a multiline keep on changing depending on evolving of new physiological races of a pathogen.
ii. **Evolving nature of pathogen races**: A new race might can develop with time which can attack all component lines of multiline.
iii. **Development of a multiline variety**: It is more time and resource consuming process as each of the component lines are required to be developed separately and breeders are also required to keep developing new resistant lines for future*. Heterogeneity in the population complicates the seed certification process.
iv. **Performance of multiline**: Since a multiline constitutes of isogenic lines derived from one common RP hence the upper ceiling of yield or any other quality improvement is limited based on performance of RP.
v. **Problem in carrying out agronomic practices**: Agronomic practices related difficulties during the use of a heterogenous plant population cannot be overlooked (Mundt, 2002).

*This problem arises when the isogenic lines or near-isogenic lines are required to be developed to form a multiline variety.

Dynamic nature of a Multiline variety:

Multiline variety is dynamic in nature (Wilson et al., 2001) as the component lines required to be changed. With time, the component lines are liable to become susceptible. Therefore, in an ideal condition a breeder of multiline variety should keep reserve of several resistant lines in advance. So, that susceptible lines can be replaced with resistant lines.

Marshall in 1977 proposed two approaches to develop a multiline variety. *Firstly*, ***Clean Crop approach*** in which ideally all the component lines of a multiline variety is resistant to all the known physiological races of a pathogen. And *secondly*, ***Dirty crop approach*** in which none of the component lines of a multiline variety is resistant to known races of the pathogen. Although the lines in dirty approach contain resistant gene however these genes are for different races than the existing races of the pathogen in a particular area. The principle behind dirty crop approach is that component lines will be attacked but the impact of pathogenesis will be relatively less due to lack of favorable host.

Multiline varieties developed in different crops

In a study conducted to uncover potential of multiline to overcome effect of blast disease in rice crop. It was observed that, multilines showed considerable capacity to maintain phenotypic stability and endure blast at the same time without decreasing yield significantly (Castro *et al*., 2022). Multilines can be used a strategy to increase the durability of resistance in plants along with other methods viz., varietal mixtures, major genes pyramiding, employing breeding methods based on minor genes selection (Khush and Jena, 2009) . In a study conducted on soyabean crop, scholars attempted to assess the phenotypic stability and yield of cultivation of purelines separately in respect to cultivation of mixture of purelines of cultivar. And they observed that average yield of multiline and purelines were same. Besides yield, other agronomic traits also showed similarity in their phenotypic expression. The average yield of the multiline did not differ from the average yield of the lines, i.e., the multiline was stable and high

yielding. For traits other than yield, there were no differences between the averages of lines and the multiline by the confidence interval. The mixture of lines is an efficient strategy to increase phenotypic stability in soybean. In a study conducted in common bean to study the effect of multiline against anthracnose disease. The scholars found that use of mixture of lines reduced anthracnose disease spread and reduced yield loss also (Botelho et al., 2011). Generally, multilines are used in self-pollinated crops, wheat, oats. However, it can be also used in cross-pollinated crops viz., 2-way or 3-way crosses in pearl-millet showed reduced rust disease and did not affected yield adversely (Tapsoda and Wilson, 1999). Cultivar mixture was successfully used in groundnut crop with stable performance in different environment and yield (Knauft *et al*., 1987; Knauft and Gorbet, 1991).

Conclusion

A heterogenous population against a pathogen not only combat disease but also stabilizes yield. A mixture of compatible purelines or isogenic lines or genotypes developed from single or complex crosses can be blend together to constitute a multiline. It is vital to test the compatibility, durability and their impact on overall yield of all the component lines of multiline. The practical benefits of mixture population in form of multiline or variety mixture is possible only when all the component lines possess sufficient phenotypic similarity to carry out agronomic practices efficiently. Multiline is mostly practiced in self-pollinated crops, viz., Oats, wheat, soyabean; although its principle can be used likewise in cross-pollinated crops also like, pearl-millet. Uniformity and compatibility among component lines of a multiline variety assures a stable population with considerable yield and resistance against various races of a pathogen. Multiline is a sustainable solution to the problem of boom and bust cycle arising due to monoculture. A multiline variety like any other variety in agriculture has its own pros and cons. And even there are few minor limitations of multiline variety it is a valuable sustainable solution to airborne disease, diseases with evolving pathogen races like rust in wheat and oats. And it has great potential to be used in other crops too irrespective of their mode of pollination.

Author Contributions

Sharma, Aarti: Writing – original draft, review & editing; Kumar, Mahesh and Shamim, Md- editing and supervision.

Declarations

Conflict of Interest: The authors declare no conflicts of interest.

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