



Recurrent introgression as a population enrichment (RIPE) method in barley

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Abstract

The RIPE (Recurrent Introgressive Population Enrichment) method has been developed to enable barley breeders to apply the basic principles of recurrent selection to a normally self-pollinated crop. This method combines the male sterile facilitated recurrent selection population (MSFRSP) methods which have been applied to barley with the hierarchical population structure of the HOPE method developed for maize. It utilizes the tight linkage of a xenia-expressing shrunken endosperm marker gene and a recessive male sterile gene on chromosome 6H (*sex1+msg6*) for pre-sowing selection of male sterile plants. It facilitates recombination among loci within the population to produce high performing genotypes with desirable agronomic traits and disease resistance.

The basis of the RIPE system includes using genetic male sterility to produce large numbers of crosses, growth rooms and off-season nurseries for accelerating generations, efficient early generation yield trials, and rapid recycling of superior germplasm. Modifications to the original system have improved the efficiency of manipulating the male sterility, accelerated the rate of initial introgression, and increased the overall efficiency of the system in producing superior lines and cultivars.

Results of using this system to develop material with increased yield, and improved agronomic traits, seed quality, and disease resistance will be reported. Since its inception, the RIPE method has evolved into a very efficient population breeding method which emphasizes the accumulation of new desirable alleles in the Elite population while retaining the existing adapted background. Four cultivars have been developed through the system to date.

Introduction

Genetic male sterility in barley was discovered in 1936 by CA Suneson (1940) in his plots at Davis, California. Suneson immediately saw the usefulness of the recessive male sterility in reducing the labour required for hand crossing and increased crossing efficiency. Suneson and Wiebe (1962) created an 'evolutionary' composite cross population (XXI) with the possibility of massive intercrossing among male sterile and fertile plants. These uses of genetic male sterility led to the concept of MSFRP (Eslick and Hockett, 1974). The populations created through the efforts of these, and other, researchers have resulted in numerous varieties and have contributed significantly to several ongoing public and private breeding programs. Ramage (1977) applied the MSFRP approach to optimize the 'happy home' for a desired allele by recombining the background rather than backcrossing a desired allele into different backgrounds in hopes of finding a good 'fit'. Most breeding programs can be loosely described as relatively inefficient recurrent selection programs in very restricted gene bases. Long cycle times (10-15 years) and few unique ancestral genotypes limit the rate of progress in barley breeding in most programs around the world. The genetic male sterility in barley provides an efficient mechanism for the large-scale crossing, both among and within populations, that is necessary to make recurrent selection work effectively and can lead to significant broadening of the germplasm pool of a breeding program.

Kannenberg (1981) developed the HOPE (Hierarchical Open-ended Population Enrichment) system for recurrent selection in maize. This system employed a hierarchical structure to separate elite, high performing materials from less adapted material with lower performance expectations. The open-ended aspect allowed for the introduction of new alleles into the overall population, and their advancement to the higher level subpopulations as their performance improved through recombination with more adapted material. The HOPE philosophy and structure was simplified and adapted to barley, however, there were some concerns about the long-term effectiveness of the HOPE-based system for barley.

The RIPE (Recurrent Introgressive Population Enrichment) system (Kannenberg and Falk, 1995) was developed to utilize the opportunities of genetic male sterility to increase the evolutionary potential of a normally self-pollinated crop through an open-ended MSFRSP concept, and to capture the efficiencies of the hierarchical structure of HOPE in making the whole system more effective in both the short and long terms (Figure 1). The RIPE system employs a pre-sowing selection system for identification of seeds which have a high probability of growing into male sterile plants (Falk, et al, 1981), along with controlled environment facilities to facilitate crossing, and offseason nurseries to accelerate generation advance and seed increase to get derived materials into yield trials (as unreplicated F3:4 with running checks) in the shortest possible time.

Original RIPE system

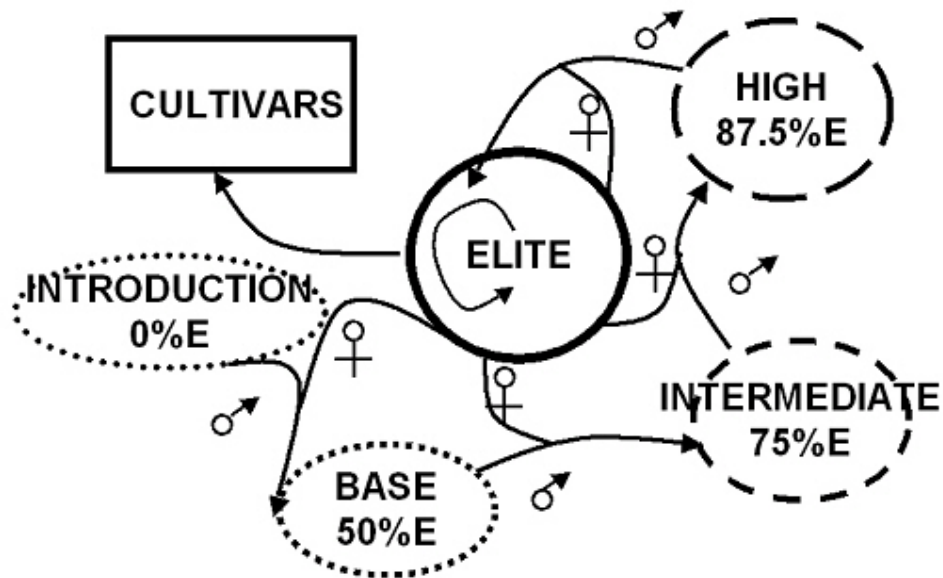


Figure 1. The original RIPE system for introgressing new germplasm into the adapted elite core of breeding material (after Kannenberg and Falk, 1995).

Population improvement by introgression of exotic germplasm into an adapted population has been shown to be effective in improving yield and quality traits of barley by Peel and Rasmusson (2000). They stressed that the existing favourable gene combinations must remain intact while introgressing small amounts of doner germplasm as this allows the potential value of the doner genes to be expressed.

Materials and Methods

Although based on the principle of the HOPE, the RIPE system was devised with increased emphasis on the introgression of new alleles into the Elite population rather than recombination among materials at each level of the hierarchy. The objective of this revised approach was a greater preservation of the well-adapted Elite gene pool by limiting the proportion of new alleles entering the Elite level, and a greater likelihood of new, desirable alleles being incorporated into the Elite population by being expressed in an increasingly Elite background in the process of advancing through the hierarchy. By recurrently crossing with Elite material at each step, new alleles are being incorporated into backgrounds which have a greater probability of being adapted and of having high performance potential. Alleles which

complement this background favourably will be selected and those alleles that interact negatively with the background will be eliminated. By the time the new, desirable alleles are incorporated into the Elite population, they will carry very few undesirable alleles with them. Because the advanced lines with new, desirable alleles are in an increasingly Elite background, they will not 'dilute' (nor 'pollute') the Elite gene pool with an excessive number of undesirable alleles nor epistatic interactions—they will also be in an Elite 'happy home'. They will already have the basic constellation of alleles needed for adaptation and performance in the target environment(s).

While some desirable alleles may be lost along the way, those with major positive effects should be retained and will be incorporated into the Elite population where they should soon replace their less desirable allelic forms through the recurrent selection practiced within the Elite population itself. Thus, there should be new opportunities for evolution to occur in every cycle as alleles are circulated within the Elite population. However, the proportion of new alleles will be low (about 6%), so they should not 'swamp' the existing desirable alleles for adaptation, performance, agronomic type, disease resistance, etc. Moderate selection intensity for more desirable expression of specific traits should rapidly incorporate positive alleles, while reducing the proportion of the undesirable alleles at those loci with a significant effect to low levels quite rapidly.

A recent modification of the basic RIPE system has been to re-configure the basic population structure to allow the easy elimination of heterozygosity for male sterility prior to the winter increase phase (Figure 2). This is based on the repulsion linkage of the orange lemma gene (formerly in coupling phase as an additional marker for male sterility) which gives a quick, visual identification of plants that are homozygous for the plump seed and male fertile alleles. As a result, all the plants grown in the offseason nursery should be homozygous and potential candidates for yield trials, whereas in the present system, 40% are heterozygotes whose progeny will segregate for male sterility, and which are taking up space (and \$\$\$) that could be better used for growing homozygotes. The breeding program is currently in the process of converting the Elite population to the new configuration of the marker alleles. There will be a slightly different progression of material through the system in that at the Base level, the Elite will be used as the male parent (and bring in the orange lemma allele) and shrunken seeds from the Base F2 will be used as the females. At the subsequent levels, shrunken endosperm seeds from the Elite level will be used as the source of the females in crossing to homozygous orange lemma males.

Modified RIPE system

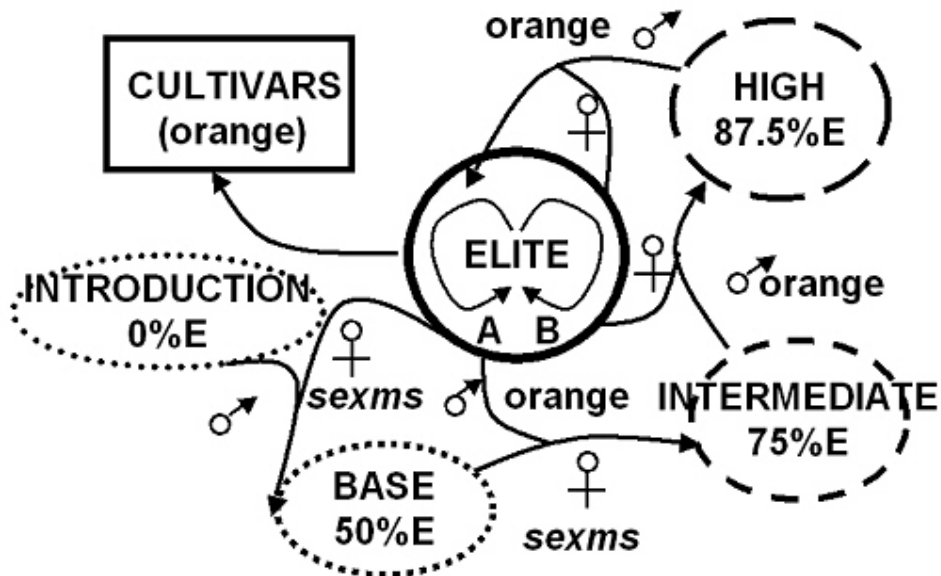


Figure 2. Modifications of the RIPE system to incorporate a marker for homozygous fertile lines (orange lemma) and separation of proven parents from new, superior male parents within the Elite level.

The Elite level has been subdivided into two sections with the 'A' group of males being from lines that are in advanced, replicated trials ('proven performers'); they will be crossed with all females in a Design 2 fashion. The females for the next round of crosses will be selected from this group. The 'B' group of males will be the best lines from the Elite and High levels in the current round of F4 yield trials; these are the 'new bulls' whose genetic potential is not yet proven.

Accelerating the introgression phase of the system is being undertaken with some of the 'wider' crosses that need more pre-breeding or adaptation before they can be expected to contribute to the Elite population (Figure 3). It is much harder to recognize desirable alleles when they are in unadapted backgrounds, so it is necessary to introgress them into a 'happier home' before their potential worth can be truly assessed. The F1 plants of the first round of crosses to produce the Base level are crossed onto Elite male steriles during the winter growth room generation, thus producing the Intermediate level. The F1's from these crosses are grown in the field. The F2 from fertile Intermediate plants are used as a source of male steriles (shrunken seed) for the next round of crossing, which produces the High level (now 87.5% Elite germplasm); they then follow the normal generation advance cycle. The rapid introgression has proven to be particularly useful in dealing with a series of crosses to

H. spontaneum to bring in more disease resistance. Several lines with improved lodging resistance and seed size have been identified in the High level yield trials, as well as some with greatly increased tillering and 'stay green', and some other rather unique plant types.

Accelerated and Modified RIPE system

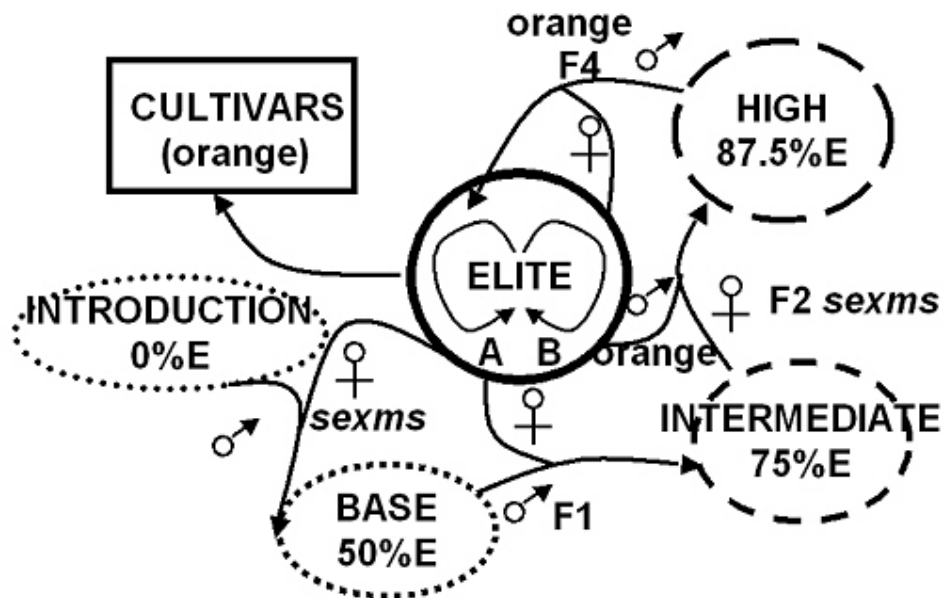


Figure 3. Accelerated RIPE system which gives yield trials (F3:4) of material at the High level (87.5% Elite) within three years of introduction to the system.

Specific-purpose subpopulations have been developed where there are special traits required/desirable for particular end uses that are unique, and that may not be compatible (nor desirable) to have circulating in the main population (ie hulless, low phytate, and semi-dwarf for swine; hooded for forage).

Kannenberg (2001) has recently proposed some additional changes in the HOPE system, based on analysis of the past 20 years of operation. One significant change is elimination of the Intermediate level, and another is using an Elite top cross as the method of entry of new material into the system at the Low level. This last change, in particular, brings the HOPE system closer to the introgression concept of the RIPE system.

Results and Discussion

The average yield (grid adjusted to check plot yields) of the F3:4 Elite lines tested in 2000 at the Elora Research Station was 91% of the mean of the official yield checks. The best line was 158% of the checks and the poorest line 49%. The best family (10 lines) yielded 110% of the checks. The average of the best line in each family was 110%. The average yield of lines at the High level was 86% of the yield checks with a range of 113%-42%. Lines at the Intermediate level averaged 96% of the check yields, with the highest line being 185% of the checks. The best Intermediate family was 120% of the check yields. Many of the lines at this level had complete resistance to both powdery mildew and leaf rust, which gave them a decided yield advantage over the checks, and also over the higher levels of the RIPE system. These results help to emphasize the significance of disease resistance and adaptation in realizing the full yield potential of lines.

Lines selected from the Elite level in 1999 and entered in replicated yield trials in 2000 (F3:5), averaged 109% of the official yield checks at the Elora Research Station with a moderate epiphytotic of both powdery mildew and leaf rust during the growing season. The best line yielded 145% of the check mean and 69% of the lines tested outyielded the checks. 16% of the lines had complete resistance to both leaf rust and powdery mildew, while only 4% were susceptible to both; the remaining 80% were moderately resistant to one or both diseases. The best check was moderately susceptible to both diseases; all registered cultivars are susceptible to leaf rust at this time. The kernel weight of the Elite lines was 9% higher than the checks and test weight was 3% higher. Lodging in the lines was 65% less than the checks and the lines were one day earlier in heading. Thus, there were significant increases in yield, along with grain quality, agronomic type, and disease resistance in the Elite-derived lines. Most other breeding programs, both private and public, are now using our RIPE-derived advanced lines in their breeding crosses for disease resistance and agronomic type, as well as yield.

A project is currently under way to improve the lodging resistance of barley by evaluating the physical attributes of the barley straw which are related to lodging resistance. Selection among lines in the Elite level has given a wide range of lodging resistance, and the physical traits associated with it; they are being recombined to initiate the next cycle of selection.

Four cultivars have been released from the RIPE system. The one which is in commercial production, OAC Baxter, was the second highest yielding variety in Performance Testing across the major barley growing regions of the province in 2000 and has significantly improved test weight and kernel weight, and good lodging resistance. Lines derived from crosses with OAC Baxter are already in replicated yield trials.

Conclusions

Applying the RIPE methodology to breeding barley in Ontario has resulted in significantly improved yield and agronomic traits, along with better disease resistance and grain quality, while reducing the size of the program. The RIPE system is a methodology which is based on preserving the basic core of adapted germplasm while integrating new, desirable traits into the active gene pool in a systematic and efficient manner. Because of the diversity of the Elite population, it is a good population in which to start selection for new traits, and then introgress more extreme types into it without losing the basic background.

The basic method should be useful in addressing any specific traits where there are good selection methods and sufficient genetic variability, while maintaining, and probably improving, the general background phenotype as well. It should also work well in other self-pollinated crops where genetic male sterility is available, and where accelerating generations is possible.

Acknowledgments

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