Review Article

CONCEPT AND RATIONALE OF EVOLUTIONARY PLANT BREEDING AND ITS STATUS IN NEPAL

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ABSTRACT

Nepal has released and registered a total of 623 genetically uniform (mono genotyped) varieties. These varieties were developed by both conventional and classical plant breeding, biotech-assisted plant breeding, and participatory plant breeding methods. However, these varieties have been shown to vary in their yield performance over the years and locations. Smallholder farmers dominate agriculture with 53% of the land-owning households with their land holding size of less than 0.5 ha in Nepal. Farmers are increasingly losing their own saved seeds. There have been impacts of weather variability, often modern crop varieties are not available to suit with these changing conditions. Farmers are looking for crop varieties that can better adapt to these changing conditions, and seeds of which can be saved for the next season planting. Evolutionary Plant Breeding (EPB), which creates and maintains a high degree of genetic diversity (i.e. polymorphic population), is a choice for breeders and farmers for accelerating the development of climate resilient and sustainably high-performance crop varieties. In 2015, the National Gene Bank in Nepal started an EPB program for the local rice variety, Jumli Marshi with the objective of enhancing genetic conservation through creating a dynamic gene pool. An evolutionary population can be compared to a living gene bank, not only in line with bringing greater yield stability, but also greater diversity in aroma, nutritional value and quality. Evolutionary populations have the potential to produce higher yields and perform better than their local or improved counterparts in adverse, or stress conditions. Under stress conditions, evolutionary populations have also been shown to be more resistant to weeds, diseases and pests damage than homogenous crop populations. Based on the source of diversity used in EPB, two different types of populations- Composite Cross population, and Composite Mixtures, population are developed. With the exception of Europe, and only for some crops, existing seed policies do not favor such populations. Therefore, there is a need to revise seed regulations in order to allow the cultivation of a higher degree of genetic diversity.

Key words: Adaptability, composite variety, diverse genotypes, evolutionary population, mixture

INTRODUCTION

Nepalese agriculture is characterized by very small land holdings scattered amongst different plots, not well adapted to high input agriculture. Land owned by farmers is their most important economic assets for food and nutrition security. 65% of Nepalese population is engaged in agriculture (MoAD, 2013), with an average size of land owned by the household of only 0.68 ha (CBS, 2013). Three percent of households are landless, 10% of the land-owning households have less than 0.1 ha, and 53% of the land-owning households have less than 0.5 ha (CBS, 2013).

Nepal is rich in crop agrobiodiversity, with over 30,000 crop landraces, mainly developed and maintained by local communities due to different climates and altitudinal variation as well diverse socio-economical settings. A total of 623 crop varieties have been released and registered to date; among them 3 were developed through participatory plant breeding, 10 through biotech assisted plant breeding, and the remaining 610 through conventional plant breeding methods (Joshi et al., 2017b). Nepal has released and registered only mono genotyped (genetically uniform) varieties; however these varieties have been found to vary in their yield performance over years and locations. Similar variation has been observed in Europe for wheat, a crop for which uniform varieties are predominantly grown. Findings of a recent study revealed that the yield variability associated with weather fluctuations can range from 15 to 75%, depending on the region (Ray et al., 2015).

About 50% of local crop varietal diversity in Nepal has been lost mainly because of wide spread use of modern uniform varieties (Upadhyay & Joshi, 2003; Joshi et al., 2020a). At the same time, farmers have experienced complete failure of production with the use of some modern varieties, while this has not been reported in the case landrace production. The impact of climate changes, particularly, weather variability, has been felt by farmers, who are looking for sustainable alternative options adapted to their low-cost agricultural system. Cultivating different crops, different varieties, and landraces of the same crop, and heterogeneous varieties in small pieces of farmland are common practices followed by smallholder farmers in Nepal to retain and maximize adaptability and stability of yield over time and space. This practice does not only provide diverse food, which has been shown to be beneficial to health (Ceccarelli, 2019), but also conserves biodiversity on-farm.

Classification and features of plant breeding

We broadly classify plant breeding into two categories, (1) conventional or classical plant breeding together with biotech assisted plant breeding including newer genetic engineering approaches and molecular breeding and often referred to as modern breeding (Fu, 2015; Baloglu, 2018), and (2) participatory crop improvement approaches including participatory plant breeding (PPB), and evolutionary plant breeding (EPB). Conventional or classical plant breeding includes old and widely used techniques of simple selection or crossing followed by selection e.g. pure line selection, pedigree selection. Today, conventional breeding also includes method that uses more recent biotechnological tools, including genetic engineering. Participatory crop improvement methods are based on decentralized selection and involves farmers in the entire breeding process. However, participatory plant breeding (PPB), as with the more conventional breeding processes presented above all produce uniform variety that suits for large-scale commercial and mechanized production, even though PPB could, in principle, also develop heterogeneous varieties (Ceccarelli & Grando, 2007). On the contrary, Evolutionary plant breeding (EPB) unlike other participatory or conventional methods, does not aim at producing homogeneous varieties, but heterogeneous populations. Evolutionary breeding can have different levels of farmer participation and therefore it may be participatory EPB, or simply EPB. Comparative analysis of these four breeding types is given in Table 1.

Table 1. Comparative analysis of broad categories of plant breeding

SN	Feature	Conventional / plant breeding Approaches		Participatory Crop Improvement Approaches	
		Classical plant breeding	Biotech assisted plant breeding	Participatory Plant Breeding (PPB)	Evolutionary Plant Breeding (EPB)
	Parental lines used	Few	Few	Few	Many
	Breeding site	On-station	On-station	On-farm	On-farm or on-station
	Testing site	On-farm (breeder led)	On-farm (breeder led)	On-farm (farmer led)	On-farm (farmer led)
	Major role	Breeder	Breeder	Breeder and farmer	Breeder and farmer
	Genetic make up	Uniform	Uniform	Uniform/ heterogeneous	Multiform
	Buffering capacity	Low	Low	Low /High	High
	Evolutionary process	Arrested	Arrested	Arrested	Continue
	Diversity conservation	Low	Low	Medium	High
	Farmers adaptation of new variety	Low	Low	High	High
	Effectiveness of selection over the year	Low	Low	Medium	High
	Duration	Long	Short	Medium	Medium
	Cost	Medium	High	Low	Low

Source: Joshi, 2017; Ceccarelli & Grando, 2019; Raggi et al., 2017; Fu, 2015; Breseghello, 2013; Moose and Mumm, 2008; Witcombe et al 2005

Within these four classes of plant breeding, different breeding terms have been coined and implemented in many different ways, for example, analytical breeding, backcross breeding, combination breeding, genetic engineering, heterosis breeding, inbreeding, introgressive breeding, landrace enhancement and conservation (LEC), maintenance breeding, molecular breeding, multiline breeding, mutation breeding, participatory plant breeding PPB), polyploidy breeding, quality breeding, resistance breeding, shuttle breeding, space breeding, stress resistance breeding and transgressive breeding. Majority of these methods target to develop uniform varieties.

Regardless of how it is implemented, the end product is a uniform variety or population, which has low buffering capacity under changing conditions (Figure 1). It is natural phenomenon that climate, genotypes and different factors keep changing a world and this might be the one reason of poor adaptation of uniform varieties. On the other hand, evolutionary populations as the end product of EPB have high buffering capacity and can easily adjust their genetic make up to adapt to different locations and changing climatic conditions, mainly because of higher evolutionary rate (Figure 2).

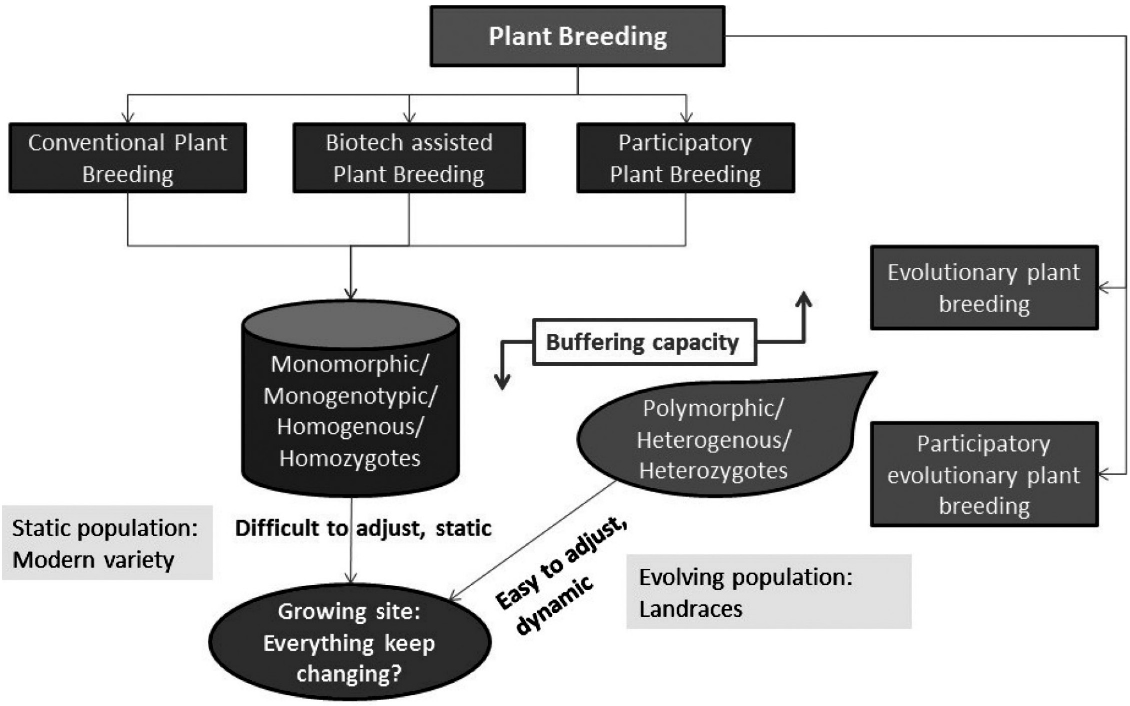

Figure 1. End products of different breeding methods and their major features

Evolutionary plant breeding

Concept and rationale

In 1956, an evolutionary plant breeding (EPB) method was proposed by the American agronomist Coit Suneson who also conducted experiments with barley evolutionary populations (Suneson, 1956). The method was based on the creation of composite cross populations by assembling seed stocks with diverse evolutionary origins, recombination of these stocks by hybridization, the bulking of F_1 progeny, and subsequent natural selection for mass sorting of the progeny in successive natural cropping environments. This EPB, however, has not been widely used and recently, it has been realized importance for developing climate resilient dynamic populations that also help to conserve genetic diversity (Rahmanian et al., 2014; Raggi et al., 2017; Ceccarelli, 2009).

Indeed, Evolutionary Plant Breeding (EPB) is a practice that allows consideration of an even wider genetic base, which favors increasing the rate of evolution of the crop population, promoting use and conservation of crop diversity and developing dynamic population to cope with change. Evolutionary Populations (EP) of crops can adapt to climate change and heterogeneous agro-ecological environments, increasing farmers' resilience and enhancing on farm biodiversity (Petitti et al., 2018). EPB create and maintain high degree polymorpho typic populations for accelerating development of climate resilient and sustainably high-performance varieties (Ceccarelli, 2009). Evolutionary populations (EPs) of maize, barley, bread wheat, durum wheat, common bean, tomato and summer squash are currently grown in Jordan, Ethiopia, Iran, and Italy (Ceccarelli, 2017). EPB can also be applied where there is a lack of crop breeding programs, specifically in addressing the needs of organic low input agriculture, high stress environments, and limited access to institutional support.

In 2008, EPB was first implemented in barley and wheat in a formal project supported by the International Fund for Agricultural Development (IFAD) in Iran (Rahmanian et al., 2014).

Despite late sowing, the evolutionary populations of barley out-yielded the local barley and performed at par to those improved barley cultivar (Rahmanian et al., 2014). In the following year, the evolutionary populations of wheat yielded more than twice as much as the local varieties. Iranian farmers have marketed the bread obtained from growing an EP of wheat (Ceccarelli, 2017). The bread wheat EP has greater yield stability, and bread obtained from its flour has a better aroma and taste. EP has been done in Italy on zucchini by intercrossing 11 commercial hybrids. Farmers growing evolutionary populations have reported higher yields, lower weed infestation and disease presence, and with lower insect damages (Rahmanian et al., 2014).

In 2015, the National Gene bank of Nepal started an EPB program in Jumli Marshi rice with the aim of enhancing conservation through creating a dynamic gene pool. EPB maintains and creates a high level of genetic diversity in crop populations by planting a much larger mixture of different varieties, relying mainly on natural selection, and does not necessarily aim to arrive at single variety.When EPB involves active participation of farmers on farm with limited influence by plant breeder throughout the breeding process, it becomes decentralized-participatory EPB. All the alleles in a population are under natural selection and evolve naturally depending on their association with fitness (Döring et al., 2011). Those individuals within a population, which increase their frequencies in the subsequent generations under the pressure of natural selection, are generally considered naturally fit. In addition to its effectiveness in coping with the changes in both biotic and abiotic stresses due to climate changes, it manages agricultural biodiversity through use, conservation and maintenance of dynamic plant populations, continued evolution and natural selection (Rahmanina et al., 2014; Raggi et al., 2017; Phillips & Wolfe, 2005; Ceccarelli, 2017).

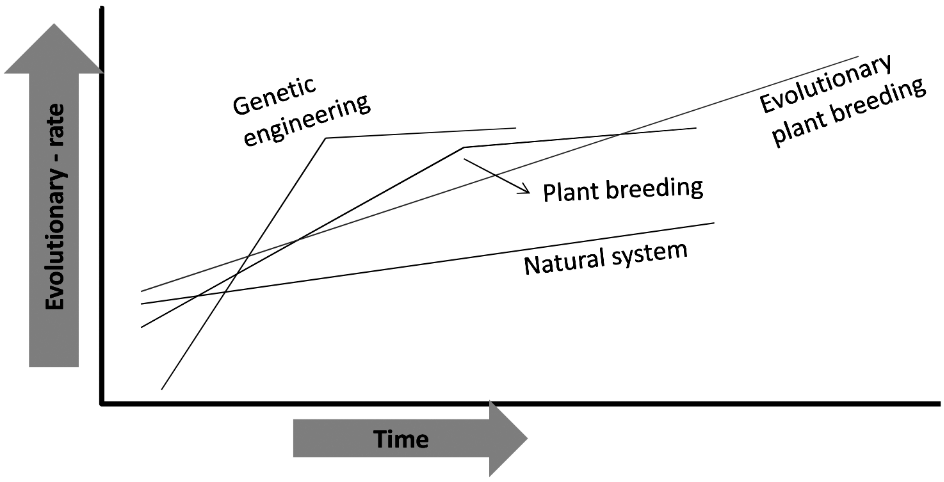

Figure 2. Hypothetical evolutionary rate of different breeding methods over time

EPB method

EPB is a simple and inexpensive method for developing broad genetic base population or variety (may be called composite or pool variety). Mixing diverse genotypes (10, 20, 100 or 1000 or even more) and growing together in a larger plot is the main method of EPB followed globally (Raggi et al, 2017; Ceccarelli, 2009; Suneson, 1956). Components of population may be landraces, improved varieties, hybrids, segregating lines, breeding lines, wild relatives etc. EPB is equally applicable to self-pollinated, cross-pollinated and vegetative propagated crops. As presented in Figure (3), the general method of EPB includes stable lines or segregating lines developed through conventional or modern plant breeding methods which can be bulked without selection, or selected individual plants (lines) can be mixed at the end. A heterozygotes and heterogeneous population, or “variety” is the product of EPB, and the variety is released for general cultivation at the end of breeding program. Farmers can continue growing the evolutionary population variety over the years by keeping mix seeds from their own field without purchasing seeds from market. This can help to overcome the monopoly of market seeds, giving small holder farmers additional options to improved single variety and hybrids. Detail steps of EPB are described below (Figure 3).

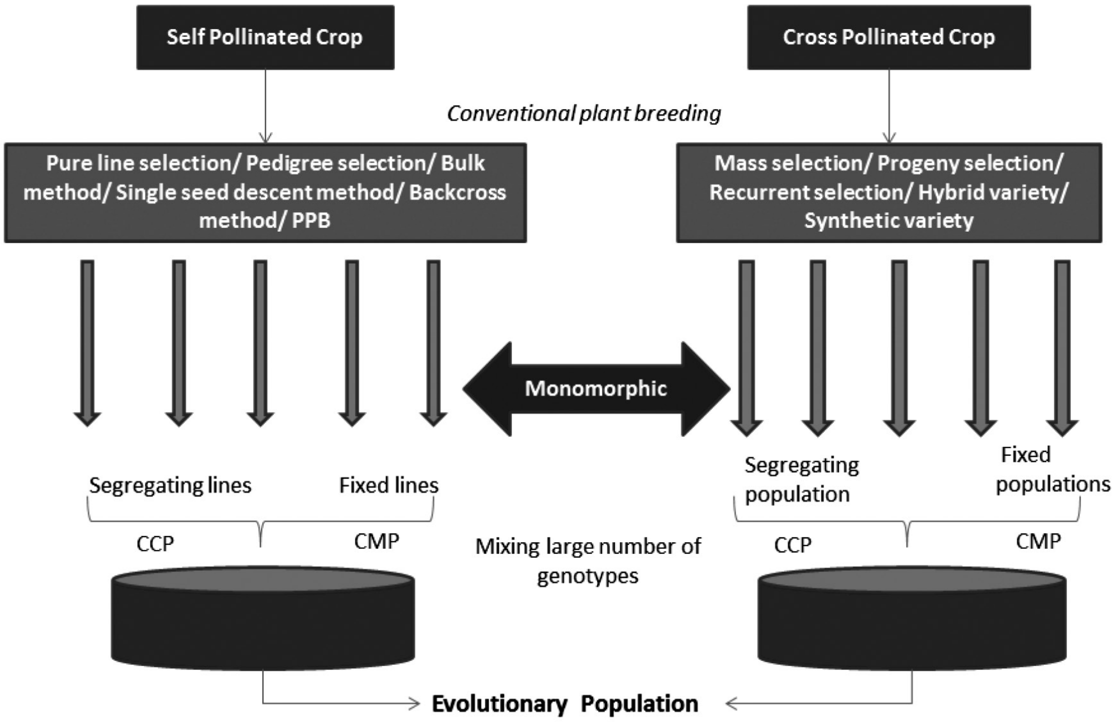

Figure 3. Methodological description of EPB following conventional plant breeding methods, CCP, composite cross population; CMP, composite mixture population

Steps of evolutionary plant breeding

General steps of EPB are presented in Figure (4). The first step is the setting of criteria for selecting or creating crop genetic diversity based on the objectives. True EPs can be developed either mixing stable genotypes or the products (F_1 or segregating populations) of multiple crosses. In second step, crop diversities are selected, collected and/ or created. Proportionate mixing of seeds of these selected varieties is the third step. Fourth step can be repeated over the times to advance the population following EPB principle. At later stages, conventional plant breeding steps can be adopted i.e. testing EPs in farmers' fields, releasing, seed multiplication and dissemination. Throughout the breeding program, priority is given to natural forces on selection with limited influence of plant breeders, while selection of seeds for the next generation planting is based on farmer preferred traits. During initial steps of EPB, negative selection (extremely undesirable genotypes are not harvested, which removes the deleterious rare alleles from evolving population) is applied and seeds from all different types of genotypes are harvested and mixed for next season growing.

Types of evolutionary population

Crop populations that are evolving can be developed by evolutionary plant breeding, participatory evolutionary plant breeding, multiline varieties, cultivar, or genetic mixtures and cross-pollinated crops. Based on the source of diversity used in EPB, there may be two different types of populations. These include, Composite Cross Populations (CCP, and Composite Mixture Populations (CMP) (Figure 4). CCP are developed through mixing different segregating lines that provide dynamic gene pools, which in turn provide a means of conserving germplasm in addition to providing selection ground (Phillips &Wolfe, 2005). Composite mixture population (CMP) is simply the physical mixing seed of existing varieties. The frequencies of different genotypes in the population change from season to season, depending on the genetic variation available and the strength and direction of environmental variables.

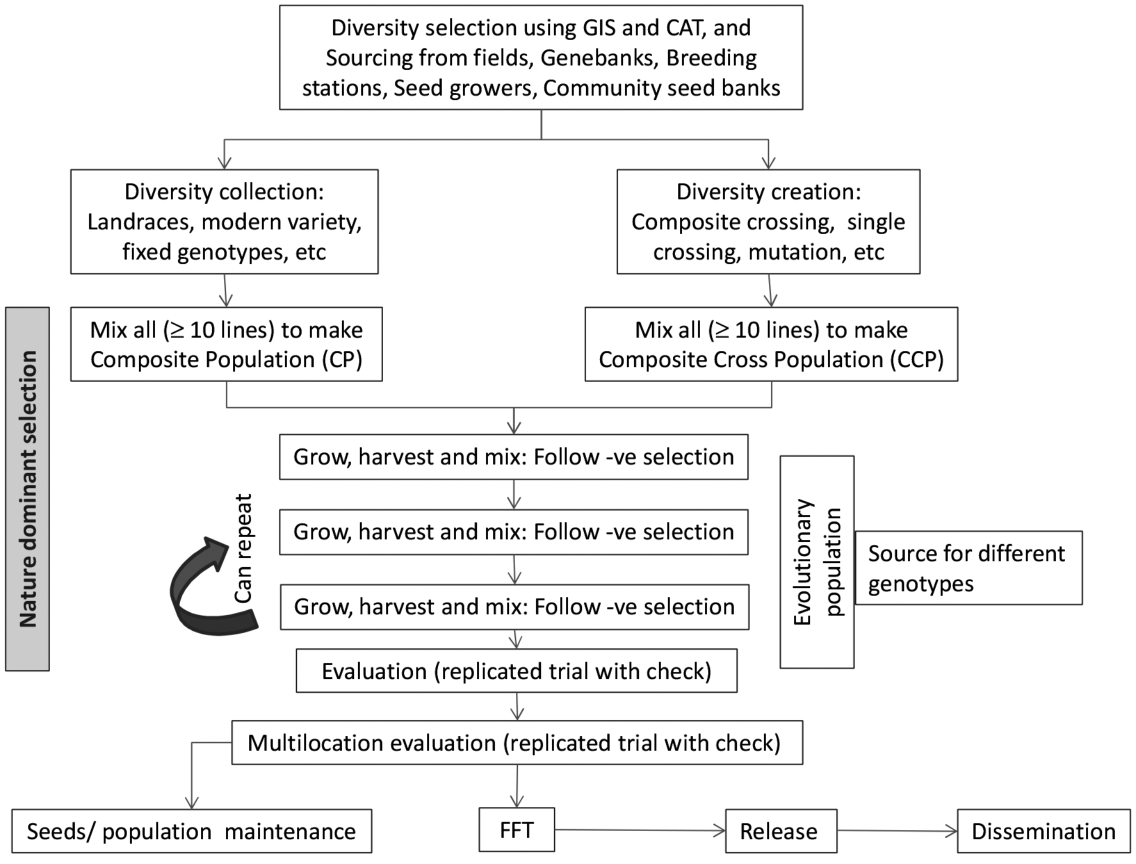

Figure 4. General steps of evolutionary plant breeding (either on-station, or on-farm i.e. participatory evolutionary plant breeding)

Sources of genetic materials

Cultivars can be sourced on-farm i.e. landraces, from Gene bank (landraces and released varieties), from research station (breeding lines, released varieties and segregating populations), and from community seed banks (local landraces).

Identification of genetic materials

Genetic materials for EP can be selected using climate analog sites and breeding objectives. Farmers are integral to the selection process to ensure the final product is adapted to their needs. Varieties can also be identified based on the similar cultivating domains. For example, in Nepal, several varieties have been released for cultivating in the same environments. GIS/CAT (geographical information system/ climate analog tool) can be used to identify the analog sites and potential list of landraces. In addition, materials can be identified based on literature reviews and site visits where local varieties are currently being grown. Materials with diverse functional traits adapted to local farmer preferences can enhance the creation to create a EP. Diverse parental lines in terms of target traits should be identified for developing composite cross population.

Important traits in evolutionary populations

For mixing cultivars, traits combination is a major consideration. Discussion among farmers and breeders should be made on selecting germplasm based on different traits targeting to minimize abiotic and biotic stress. Different contrasting traits need to be considered, while ensuring, traits for all mixing germplasm should have similar maturity, cooking time or method, and milling quality. Complementary traits for vertical and horizontal spaces need to be considered, including different root length, plant height, plant structure, and plant shape and size (Table 2). For disease and insect pests, important traits are: different reaction capacity with insect pests and diseases, leaf and stem texture, color and size, and scent, secondary metabolites. Traits related to drought management are deep root, erect plant/leaf, different plant height, and large leaf but few in number.

Table 2. Criteria and traits for selecting cultivars based on objectives to mix

For space use	For disease and insect pests management	For drought management	Trait to be similar among components
Different root length	Different reaction capacity with insect pests and diseases	Deep root	Maturity*
Different plant height	Different leaf and stem texture	Erect plant/leaf	Cooking method
Different plant structure	Different color and size	Different plant height	Milling
Different size	Different scent	Large leaf but few in number	Threshing
Different plant shape	Different inflorescence	Shiny and rough leaf	Cooking time

Note: * If hand picking is preferred, and nature of harvest is multiple times, then different maturity period can be considered.

Evaluation of EP: Evaluation methods commonly used in conventional breeding can be applied to EPB. Broadly three evaluation categories should be considered for evaluating EP. The first one is sensory evaluation, which includes eye determination, and tongue and skin determination. Farmers and breeders should be invited at later stages in the process for such type of evaluation during different crop stages (e.g. mid flowering time, maturity time, etc.). Second, is measurement-based evaluation, which includes evaluation over the years against base population and landrace/ local check (trend analysis) on target traits and adaptation traits. Researchers should look for new genotypes in the population. Third one is adaptation study which is the major part to evaluate EP. Adaptation should be based on farmers' preference as well as environmental performance.

Cultivar (genetic) mixtures and EPB

Cultivar mixtures or genetic mixtures are mixtures of cultivated varieties (generally few in number) growing simultaneously on the same parcel of land with no attempt to breed for phenotypic uniformity (Mundt, 2002). The importance of cultivar or genetic mixtures has been well demonstrated for rusts and barley powdery mildews of cereals (Mundt, 2002). Multiline cultivars of rice are widely used to prevent the breakdown of resistance against blast in Japan, where the first registered rice multiline was released in 1995 (Koizumi, 2001). Mixing existing cultivars with more diverse genetic backgrounds than multi lines can enhance functional diversity and improve yield by providing more chances for positive interactions among cultivars. The success of the large-scale farmer participatory experiment in Yunnan, China, was of mixture of traditional cultivars and hybrid rice inter-planted to control blast and increase grain yield (Zhu et al., 2000).

Cultivar mixture is a simple and sustainable genetic resources management system. It helps to increase yield, provide yield stability, conserve genes, manage diseases (buffer against disease loss), and restrict the spread of disease considerably. Functional diversity leads to higher stability (Petchey & Gaston, 2002). These mixtures support prolonging the useful life of resistance genes and increasing crop productivity by taking into account the functional differences in disease resistance and other agronomic traits of cultivars.

Successful Cases: Cultivar mixture and EPB in Nepal

Cultivar mixtures in Nepal

Mixture cultivation is commonly used by many farmers in Nepal (Joshi et al., 2017c). Farmers grow several cultivars in a field or adjacent field as a strategy to cope with heterogeneous and uncertain ecological and socioeconomic conditions. Varietal mixture research in Nepal began in 1995 using barley, targeting to develop populations that control yellow rust and produces higher yield (Pradhanang & Sthapit, 1995). In wheat, mixture response was studied against spot blotch (Sharma & Dublin, 1996). Bi-blends of nine rice genotypes were studied for blast control in 2005, particularly to estimate the mixing ability. Competition effects were also estimated among wild rice species, F_1 hybrids, released varieties and landraces (Joshi et al., 2017a). In 2015, a rice mixture trial was done aiming to control the blast, and bean mixture to control anthracnose and rust in Jumla district (Joshi et al 2020b). Mixture plots of both crops had less disease infestation. Seed setting problem was also partly solved by mixing common buckwheat landraces (NAGRC, 2018).

EPB in Nepal

One of the conservation strategies of the Nepal National Gene bank is use of evolutionary plant breeding approach for conservation of intra-landrace diversity on-farm. Jumli Marshi, one of the cold tolerant and popular rice landraces in mountain ecosystem was targeted for EPB. One of the major problems in Jumli Marshi is blast disease. EPB targeted to minimize the loss of grain yield due to by blast infestation, by developing an enhanced broad genetic base for Jumli Marshi. Collections of Jumli Marshi rice landraces (about more than 10 accessions) were mixed (called composite mixture population) and were grown in the field. Distinct lines within a plot were identified based on the important morphological parameters. After negative selection within each type, these lines were mixed and were planted in the field for further selection. This process has been continued since 2015 and population is now maintained as blast free with higher grain yield (about 10% higher than original population).

From 2018 onwards, National Gene bank has started participatory EPB in rice and bean in collaboration with Local Initiatives for Biodiversity, Research and Development (LI-BIRD), and Bioversity International (BI), with the financial support from International Fund for Agricultural Development (IFAD). Jumla and Lamjung are the sites for EPB on rice whereas Jumla is also a site for EPB to work on bean. Composite mixture populations of both rice and bean were developed using GIS and CAT, and components (genetic materials) were collected from farmers' fields, community seed banks, the Nepal National Gene bank, and research stations of Nepal Agricultural Research Council (NARC). Accordingly, four different evolutionary populations have been planted for bean and rice in Jumla and Lamjung districts.

The first bean evolutionary population consisted of 25 local landraces, collected from Jumla district. The second one consists of 40 landraces from similar agro-ecological domains across Nepal; the third bean EP consists of 12 breeding lines, and one released variety, and the fourth bean EP consists of 78 components, including mixture of all local landraces, breeding lines, and released varieties. Rice EP experiments were established in two sites, in Jumla district, and Lamjung district. In the case of rice EP establishment in Jumla district, the first EP consisted of 4 local landraces, the second EP consisted of 37 local landraces from similar agro-ecological domains across Nepal; the third EP consists of 5 released varieties, and the fourth EP consists of 66 components, including landraces, improved varieties, and breeding lines. In Lamjung district, the first rice EP consists of 21 local landraces, the second rice EP consists of 25 landraces from similar agro-ecological domains across Nepal; the third EP consists of 5 released varieties, and the fourth EP consists of 56 components with mixture of landraces, released varieties, and breeding lines.

For both rice and bean EP establishment and field evaluation in sites, one local landrace, check variety, and one standard check variety, was used in the trials, with two replications. While making mixture of different components in EPB trials, equal number of grains from each component germplasm sources (landraces, released varieties, and breeding lines) were mixed in rice and bean, respectively, and planted in the field.

This EPB trial has used decentralized participatory evolutionary plant breeding approach in Nepal, is now in its second year. Initial first year results show that genetic diversity is larger in the fourth population as compared to the first, second and third evolutionary population in rice and bean, based on a comparison with the number of components involved during EP establishment. At the end of EPB trial in Nepal, we can rapidly (3 to 5 years) develop EPs of rice and bean in Jumla and Lamjung districts.

Policy dimension for adopting evolutionary population

Existing seed regulations in Nepal only allow uniform varieties, or landraces to be registered, or released. Many landraces are polymorphic and do not meet the variety registration standards. Similarly, evolutionary plant population is polymorphic in nature, and therefore, there is no provision of legalizing such population for general cultivation in the country. Therefore, a system should be developed that allows for the release, or registration of crop varieties with broad genetic base. Regulations need to be revised for favoring variation of key traits at population level. In this regard, it will be important to describe the trait variation within the evolutionary population to be registered, to be defined to a level that ensures distinctness, stability, and thus certain degree of uniformity can be maintained generation after generation, once the EP is proposed for registration and release. Provision of equal proportion of each variant in a population could be the simple method for maintaining, cultivation, and distribution of population.

CONCLUSION

For centuries, conventional plant breeding has targeted the development of uniform variety having higher yield potential in favorable environment. The products of this type of breeding were not targeted to marginalized farmers, in non-uniform environments, with little access to inputs. This breeding strategy also pressured farmers to depend on seed companies to buy seeds each year. Because of their mono-morphic and uniform genetic makeup, such varieties are more vulnerable to different biotic and abiotic stresses, particularly, under changing climatic conditions. Landraces are more resilient to heterogenic and changing environmental conditions than modern variety, mainly because of high level of intra specific diversity among and within landraces. In the context of climate change and a need to reduced increased dependency, seeds company monopolies, evolutionary population is an innovate and proven option, particularly in marginal low input farming systems. Such population assures yield and a high degree for increased yield, and adaptability of the evolutionary population each year. The final product of EPB can be termed as composite, pool, or dynamic varieties. Evolutionary plant breeding can be equally applied to self-pollinated, cross-pollinated and vegetative propagated crops.

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