

CEREAL VARIETY MIXTURES AND POPULATIONS FOR SUSTAINABLE AGRICULTURE: A REVIEW

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Abstract

Modern varieties developed under conventional plant breeding programs do not always perform well under organic and low-input growing conditions. Therefore, organic farmers need varieties adapted to variable environmental conditions. This means emphasis on traits such as nutrient uptake and use efficiency, good weed suppression ability and disease resistance and can be achieved via extended genetic diversity within the varieties: variety mixtures and populations of self-pollinating crops.

The aim of the present study was to review published scientific literature about importance and research results on this topic. Scientific articles about the necessity of such studies and results of investigations performed in different countries have been studied. The main results show that variety mixtures have advantages in terms of disease control and yield stabilization and performance of mixtures may be affected by various factors – choice of components and its number, interactions between plants of components, manner of the mixing and growing technology. Depending on parental material, populations contain greater genetic diversity than variety mixtures. There are trends observed in the studies that yield of populations increased over generations and they are more stable than modern varieties, but there is no evidence that they would be significantly more productive.

This research direction has recently become topical and is not sufficiently widely covered in the world agricultural science. The obtained results are inconsistent.

Key words: variety mixtures, composite cross populations, genetic diversity.

Introduction

Genetically uniform varieties produced from simple crosses in barley (*Hordeum vulgare* L.), wheat (*Triticum aestivum* L.) and other self-pollinated crop species, give both high and stable yields, and such varieties dominate in commercial production (Soliman & Allard, 1991). However, these varieties are not high yielding by themselves. Such varieties can provide high yield under conventional growing conditions with applications of mineral fertilizers and pesticides, but do not have an ability to adapt to unstable environmental conditions, compete with weeds and effectively uptake nutrients from the soil if their amount is limited (Wolfe *et al.*, 2008).

Demand for agricultural products grown without chemically synthesized substances is increasing in the world (Wolfe *et al.*, 2008) and human activities are now increasingly influencing changes in global climate, and availability of non-renewable resources is decreasing (Chakraborty & Newton, 2011). Considering the above mentioned, it is necessary to understand which strategy is the most appropriate for the future of agricultural production (Østergård *et al.*, 2009). One of the factors in the changing environment for sustainable agriculture is that wider adaptation is needed, and that this can be achieved via extended genotypic diversity of plants within variety (Döring *et al.*, 2011; Tooker & Frank, 2012).

Increasing genetic diversity in crops can ensure yield stability and adaptability, reduce disease distribution and improve competitive ability with

weeds and nutrient uptake efficiency (Döring *et al.*, 2011). Significant positive effect of the diversity level (pure line varieties – variety mixtures – composite cross populations) was shown on grain yield, yield stability and several other traits in winter wheat (Döring *et al.*, 2015). One of the ways to increase genetic diversity of varieties is growing of variety mixtures (Lopez & Mundt, 2000). Another solution for increasing genetic diversity in varieties of self-pollinating cereals is creation of composite cross populations, which include higher levels of diversity if compared to pure lines and mixtures and are able to develop itself in time by adapting to the respective environment (Wolfe *et al.*, 2008).

European commission noted that genetic diversity in agriculture is associated with a wide range of environmental and economic benefits, which are essential for sustainable agricultural production and paid attention to practice orientated activities including action on investigating agricultural genetic resources (European Commission. Report SWD (2013) 486 final).

The aim of the present study was to summarize the results of research and potential benefits and drawbacks of the use of cereal variety mixtures and populations in the process of the agricultural production.

Materials and Methods

Monographic method has been used for this review. Scientific literature from different journals, monographs and PhD thesis has been used in it.

Literature includes information about the necessity for such studies and information from investigations performed in Latvia, Poland, Scotland, England, Denmark, France, Canada, USA, Slovakia and China.

Results and Discussion

Variety mixtures: yield and its stability

The impact of breeding on grain yield over the years was confirmed by the results of study on barley adaptation using 188 landraces, old genotypes and current modern varieties. It was concluded that landraces were generally better adapted to stress prone environments, while old and modern varieties to non-stress environments, and current barley selection is leading to specifically adapted genotypes (Pswarayi *et al.*, 2008).

One of the ways to increase adaptability to environmental stress factors is to use variety mixtures. It is a relatively unexplored measure for maintaining and stabilizing yields for both organic and conventional producers (Kaut *et al.*, 2008; Kiær, Skovgaard, & Østergård, 2012; Zhou *et al.*, 2014). Yield of cereals is forming gradually during vegetation and depends not only on variety genotype but also on environmental conditions. It is not possible to predict what mixture component will provide advantage in the respective year and environment while sowing variety mixtures, but compensation mechanism can provide stability (Kaut *et al.*, 2008).

The results of the research confirm that growing variety mixtures may have several advantages over pure crops under both organic and conventional farming systems (Kaut *et al.*, 2008). For example, field trials conducted in Canada show that spring wheat variety mixtures may provide greater stability with little or no reduction in yield. Unpredictable environmental variation factors are the main impediment in choosing the right variety or variety mixture. It is unclear how many varieties should be used to compose the mixtures. In the investigation mentioned before two-variety mixtures yielded more than three-variety mixtures at one organic location, but were not different at the other organic and conventional locations (Kaut *et al.*, 2008). Other investigation carried out for two years in Canada tested 16 three-variety spring barley mixtures in a range of possible combinations of three varieties in different proportions. Selected varieties had a range of morphological and phenological differences. All mixtures gave significantly higher yields than the lowest yielding pure stand variety while no mixture had higher yield than the highest yielding pure stand. Using two methods of analysis of yield stability two mixtures were identified as having superior yield stability (Juskiw, Helm, & Burnett, 2001).

Essah and Stoskop (2002) in Canada studied two-variety mixtures performance of phenotypically

contrasting barley varieties. The results of this study indicate a possible 13-14% yield advantage of mixtures compared to pure stands means, whereas the experiment carried out in Poland including two- and three-variety mixtures shows no evidence that variety mixtures would give higher yield if compared to pure stands on one site; however, on the other site 1-15% yield increase was found (T ratwal *et al.*, 2007). In a Danish investigation, one of six tested two-variety mixtures out-yielded all of its component varieties in almost half of the 17 trial environments (Kiær, Skovgaard, & Østergård, 2012). The contrasting results show that yields of mixtures may be affected by various factors, also by the manner of mechanical seed handling during the mixing. In an investigation carried out in the UK three winter barley varieties were mixed in equal proportions but using techniques giving different patterns of plant spatial heterogeneity. Results show that mixtures mixed directly in the seeder hopper prior to sowing gave a yield advantage if compared to the average of individual components in two out of three years, while the mixture which was pre-mixed most homogeneously gave no significant yield advantage (Newton & Guy, 2009).

Mille *et al.* (2006) report that winter barley mixtures composed of four and two components performed better than average of individual pure line components and the best yielding pure stand variety was not the best contributor to the mixtures. They recommend that mixtures of two components should be screened to remove unfavorable variety pairs and those mixtures that show complementarity should be selected in order to construct mixtures of four components. Furthermore, Mundt *et al.* (1994) in Oregon, USA, compared four barley varieties as pure stands and in 1:1 two-, three- and four-variety mixtures in all possible combinations. Generally, the mixtures did not increase yield relative to the mean of the pure stands; just one two-variety mixture consistently increased yield and other two-variety mixture had consistently lower yields than the mean of the pure stands. Also Zhou *et al.* (2014) in China investigated two and three-variety mixtures of spring wheat during four years and concluded that variety mixtures tend to increase and stabilize yields. Yield stability of three-variety mixtures exceeded that of the pure varieties and consistently out-yielded the means of the respective components over different growing conditions. Similar results achieved Cowger and Weisz (2008) in North Carolina, USA, by testing two and three-variety mixtures of winter wheat. Yield stability of mixtures exceeded that of pure stands and average yield across environments significantly surpassed their respective components. Döring *et al.* (2015) showed that multi-component mixtures of winter wheat significantly out-yielded the mean of

the respective components across 12 environments by 3.6%.

Fang *et al.* (2014) in China evaluated grain yield and water use efficiency in mixtures consisting of an old landrace and a modern variety. They suggest that the use of a mixture of such varieties in a serious drought season will ensure greater availability of water after stem elongation stage, resulting in better water use efficiency and higher yields than in cultivars grown as a pure stand.

The experimental evidence of more stable yields of mixtures than the average of their pure stand component varieties may justify cultivation of variety mixtures in variable environments, such as organic farming systems. However, mixtures would be even more advantageous to farmers in general if in addition mixture yields were comparable to the highest yields of the component varieties (Kiær, Skovgaard, & Østergård, 2012).

Variety mixtures: leaf diseases

The traits controlling diseases are particularly advantageous where zero or low use of synthetic pesticides is permitted such as in organic or low input systems, respectively (Newton *et al.*, 2012; Kiær, Skovgaard, & Østergård, 2012). In mixtures initially the attention was paid to advantages regarding to reduction of leaf disease infection, which can reach a level when usage of fungicides is economically unreasonable. It reduces also the risk that resistance genes can lose the efficiency (Mundt, 2002).

Several studies revealed that variety mixtures may be used as added elements of functional diversity to reduce levels of fungal diseases and thus provide benefits to cereal production. For example, in Slovakian three year investigation, three-variety mixtures of winter wheat were tested and lower infection with brown rust (caused by *Puccinia recondite*), powdery mildew (caused by *Blumeria graminis*) and leaf spots but no differences in respect to yellow rust (caused by *Puccinia striiformis*) in comparison with pure stands was found (Muchova & Fazekasova, 2010). Tratwal, Law, & Philpott (2007) concluded that winter barley two-variety mixtures combined with different fungicide treatments can reduce the powdery mildew occurrence compared to pure stands and can constitute an alternative way of growing winterbarley, especially at low-input and organic agriculture. Newton and Guy (2009) who investigated three winter barley varieties mixed in equal proportions for three years, but in different ways to give different patterns of spatial heterogeneity, showed that most mixtures which were mixed directly in the seeder hopper prior to sowing significantly reduced rhynchosporium (caused by *Rhynchosporium secalis*), powdery mildew and net blotch (caused by *Pyrenospora teres*) if compared

to the mean of the components, while the mixture which was pre-mixed most homogeneously, gave a significant reduction in net blotch in one year only. A five year investigation in the UK compared two, three and four-variety mixtures of winter barley in different levels of tillage. Rhynchosporium infection level was decreased in most mixtures in comparison to the pure stand mean in all years and tillage conditions. In addition, three and four-variety mixtures gave around 32% less disease than two-variety mixtures (Newton *et al.*, 2012). Spring barley varieties with mildew resistance genes were used to compose three and four-variety mixtures in Scotland. Data from this study showed that reduction in powdery mildew infection was not significant compared with the component pure stand mean. There was a trend towards greater reductions at low fertilizer level (Newton & Guy, 2011) including plot size, and its interaction with fertiliser level and barley germplasm on mixture efficacy in controlling powdery mildew were investigated. Two groups of cultivars, one from cultivars grown in the UK and the other from cultivars grown in Poland, along with all their respective three-component mixtures, were grown in three field trials, one with 13.5 m² plots, one with 0.4 m² plots, and the third in intermediate size plots, which included a mixture of all the cultivars in both groups in equal size treatments of structured spatial arrangements. Another trial utilised similar structured spatial arrangements to trial 3 was carried out using a combination of cultivars with appropriate matching virulence levels (trial 4. Mundt, Hayes, & Schon (1994) in Oregon, USA, investigated 11 possible mixtures of two susceptible and two moderately resistant advanced breeding lines of barley in equal proportions in the presence of scald and net blotch. The variety mixtures restricted leaf disease development during three seasons by 12% on average. However, mixtures of susceptible and moderately resistant line reduced disease severity by 20-32%.

Variety mixtures have been suggested as a means of controlling foliar diseases in cereals, but little information is available on how to choose components for use in the variety mixtures (Mille *et al.*, 2006). Disease intensity strongly depends on a year (Tratwal, Law, & Philpott, 2007; Nemecek *et al.*, 2011) and on the particular component varieties used in the mixture (Nemecek *et al.*, 2011).

Variety mixtures: weed suppression ability

Weed suppression ability is another necessary trait for obtaining stable yields with good quality under organic growing conditions as well as other farming systems that aim to limit the use of herbicides (Hoad, Topp, & Davies, 2008). In variety mixtures interaction between plants is related not only to competitiveness

with weeds but also between the crop plants that can reduce the ability to suppress weeds. There are no sufficient investigations made yet about plant traits according to which mixture components should be selected in order to positively interact with each other and also successfully compete with weeds. It was approved that barley mixtures can be more effective in weed suppression than the varieties in pure sowings and the effect depends on the composition of the mixture (Didon & Rodríguez, 2006).

Kaut *et al.* (2008) tested two and three-variety mixtures and concluded that, although variety mixtures varied for grain yield, they did not suppress weeds better than their pure components. Only one from nine two-variety mixtures had low total weed biomass in plots indicating improved competitive ability. Kiær, Skovgaard, & Østergård (2012) and Kaut *et al.* (2008) observed a tendency that mixtures of varieties with varying straw length have an actual advantage in terms of weed suppression. In addition, Kiær, Skovgaard, & Østergård (2012) noticed that mixing effects within the mixtures tended to be smaller, when the level of suppression of tall annual weeds by component varieties was more diverse indicating that the yield advantage of mixing varieties is lower if one component is particularly effective or particularly poor at suppressing these weeds.

In connection with plant traits potentially related to competitive ability, Döring *et al.* (2015) found significantly higher leaf area index, straw height and head density but no difference in early ground cover in multi-variety mixtures compared to the mean of components while testing under two organic and two conventional sites.

Competition has large negative effects on yield under organic management and thus both weed suppression and high yields must be considered when choosing variety mixture components taking into account that unpredictable year dependent environmental variation factors are the main impediment in choosing the right ones. If variety mixtures are to be profitably employed by organic producers, they must be competitive enough to suppress weeds to the same degree as, or preferably more than pure stand (Kaut *et al.*, 2008).

The topic about weed suppression ability is relatively new and there are not a lot of research results available on it. Research projects are going on, but results are not yet published (personal communication with weed researcher Mg.Sc. Dace Piliksere).

Populations

Another solution besides mixtures for increasing genetic diversity in varieties of self-pollinating cereals is populations. They include higher levels of diversity if compared to pure lines and mixtures and

are able to develop itself in time by adapting to the respective environment (Wolfe *et al.*, 2008).

Depending on the number of parents and the way of crossing several population types exist: simple populations obtained by crossing only two parents and more complex populations where a larger number of parents is used. Composite cross populations (CCP's) consist of bulked diallel crosses among the definite group of parents (Brown, Caligari, & Campos, 2014).

The principle of CCP's is to intercross in all combinations a number of varieties with different useful characteristics to generate a population. This is then exposed to natural selection at field sites to allow adaptation. The objective is to generate a reservoir of genetic variation that can buffer the crop against a wide range of environmental variations, more than would be possible in pedigree line varieties or in physical mixtures based on single genotypes (Döring *et al.*, 2011). There are continuous changes in populations going on and no possibility to obtain and market constant seed material (Brown, Caligari, & Campos, 2014). The diversity of the genotypes in the population changes over the time. Starting with the maximal degree of diversity of population there is a possibility that diversity will decrease over the time, because genotypes poorly suitable to the respective environmental conditions will decrease in frequency. If environmental variation is large, diversity will remain in the population for a longer period of time because selection pressure is less severe on those genotypes performing best away from the environmental mean. Similarly, including genotypes with broader ecological tolerance will keep the diversity of genotypes longer in the population (Döring *et al.*, 2011).

Harry Harlan began to make CCP's from many diverse barley varieties originating from around the world in 1920. For example, composite cross II (CC II) was a population developed by Harlan in 1929 using 28 varieties crossed in all possible combinations. These populations were planted and harvested each year under standard agronomic conditions over a period of 50 years. No artificial selection was conducted, but the populations were exposed to multiple environmental stresses. Results from numerous studies on these populations show steady increases in grain yield, disease resistance and yield stability (Hagparast *et al.*, 2010).

In 2002, three CCP's were developed by The Organic Research Centre in the UK based on 20 diverse winter wheat parents. All possible crosses of nine high yielding parents and of 12 parents selected for their milling potential were crossed to produce three CCP's: (1) high yield, (2) quality and (3) yield-quality and are extensively researched in a number of studies in different countries (Kassie, 2013; Döring *et al.*, 2015).

The effects of natural selection on grain yield of barley CCP's were investigated by Soliman and Allard (1991). They tested several generations of three populations created from a large number of parent varieties (28, 30 and more) including the previously mentioned CC II. This population was compared to six commercial varieties and showed a marked yield increase over generations, perhaps as a result of increased adaptation. The average yield over six years of the most productive population was only 85% in comparison to the best yielding commercial variety, but CCP's were in general more stable than commercial varieties. So these authors suggest if disease resistance and yield stability are two main objectives of a particular breeding program, the CCP approach is an efficient breeding method. On the other hand, if yield potential is a major goal, the CCP approach, as demonstrated by this study, is unwarranted; however, the year of creation had to be taken into account. They recommend that individual pure lines derived from the later CCP generations would be evaluated as potential breeding material and developed to varieties for low-yielding environments.

Danquah and Barrett (2002) also investigated several generations of the CCP mentioned above and developed from 30 parents. The study revealed that, on average, the control variety out-yielded the composite cross generations over the two year period in Cambridge environment. The highest yielding composite cross generation over the period of the experiment yielded only 78% of the control variety. It is interesting to note that in one season three out of nine composite cross generations performed better than control. There was a long period of drought, which probably has affected the control variety more than the composite cross populations. Kassie (2013) based on the study in a single location during four growing seasons reports that more recently created winter wheat CCP's created of nine, twelve and twenty parents showed similar yield potential as the pure line standard variety and no significant differences were observed between the generations of CCP's in the grain yield potential and yield components. Furthermore, the standard variety had better ground shading ability (Kassie, 2013), which is one of the important traits of varieties for weed suppression (Hoad *et al.*, 2008), than the CCP's. Decrease in ground shading capacity of the CCP's over the four generations was observed. Based on these results one cannot state that the CCP's are the best varieties for the organic growing conditions (Kassie, 2013). On the other hand, Döring *et al.* (2015) found a significantly higher average early ground cover, leaf area index and straw height in CCP's of the same origin in comparison to the mean of the parental varieties.

Within populations a major force is competition between different genotypes. These competition effects may limit what populations can achieve (Döring *et al.*, 2011). Kassie (2013) reported that CCP's compared to pure line variety had lower number of spikelets per spike and lower spike compactness which likely influenced yield.

Soliman and Allard (1991) concluded that the CCP's offer an opportunity to produce varieties that do not show a large genotype – environment interaction for yield because of the genetic diversity in the populations, but when they become more homogeneous, their stability is decreased. Yield advantage by 2.4% on average over 12 environments and higher yield stability for CCP's in comparison to parental mean was shown (Döring *et al.*, 2015).

Genotypically diverse populations are also known to better resist diseases, because they contain a greater range of genotypes having reduced susceptibility to pathogens; therefore, diseases will not spread that easily through the populations (Mundt, 2002). Muona, Allard, & Webster (1982) analyzed genetically changes in resistance to scald disease, caused by *Rhynchosporium secalis*, which occurred in barley CC II over 45 generations. They reported that recombination which has occurred in population has given an increase to numerous novel multiresistant genotypes. Maroof, Webster, & Allard (1983) tested the same CC II for reaction to three barley pathogens, *Blumeria graminis*, *Pyrenophora teres* and *Rhynchosporium secalis*. They concluded that many families carrying combinations of multiple resistances not observed among the parents were found in the later generations. On the other hand, Döring *et al.* (2015) did not find a significant advantage for winter wheat CCP's for foliar disease infection with *Septoria* compared to the mean of parents scored during one season in four sites.

The key for the success of populations concerns the number and choice of parental genotypes for the establishment of CCP. There are two extremes – on the one hand, one could use only two different parent varieties, on the other – as many parents as physically possible to cross with each other (Döring *et al.*, 2011). Strazdiņa *et al.* (2012) investigated barley populations originated from simple crosses and wheat populations from crosses between two or three parental varieties and concluded that yield of barley populations was in between the levels of parents and insignificant yield gain was obtained only in some cases and mostly under organic growing conditions. Some wheat populations showed a tendency to higher yield under conventional conditions; however, under organic conditions the yield of populations was between or less the range of parents. The authors explain these not very promising results by relatively low diversity of the investigated

populations than that of CCP's researched in other studies.

Some studies have been carried out to measure the necessary minimum number of parents to establish populations. Kovács (2008, cited from Döring *et al.*, 2011) reported about experience with bread wheat using different number of parents and suggest that the necessary minimum for the establishment of a successful population is around seven parents, and the optimum is around 12 parents. Döring *et al.* (2015) suggest that establishing CCP's from smaller sets of high-performing parent lines might optimize their yielding ability.

The seeds of CCP's cannot be marketed under the existing legal framework, and registration of such varieties is not possible due to non-compliance to the requirements of distinctness, uniformity and stability (DUS). However, a temporary experiment is being currently carried out in the EU allowing experimental marketing of wheat, barley, oat and maize CCP's created by crossing of five or more varieties in all possible combinations (Official Journal of the European Union. Directive 66/402/EEC).

Conclusions

1. Mixtures can easily reconstitute and they have an advantage of being legal under current legislation, whereas the trading of non-uniform

crop populations is presently possible only due to the temporary experiment providing for certain derogations for the marketing of populations.

2. Most of the studies show that mixtures have advantages in terms of leaf disease control and yield stabilization in comparison to the average of components. In respect to grain yield, it might be essential to assess the combinative ability of the components.
3. While creating mixtures, it is possible to generate much larger quantities of seed early on, whereas crop populations will need a phase of seed multiplication before they can be used on a farm. However, populations have the advantage of a much larger pool of genetic diversity.
4. Advantage of populations can be the ability to adapt to specific growing environments and thus to be unique for each farm. Populations are more likely to be superior in extreme environments like drought or low nutrient availability.
5. This research direction is not sufficiently covered in the world and reports on the results are inconsistent. Further studies about the possibilities to increase sustainability and competitive ability of the varieties by increasing the genetic diversity are needed. It is essential to understand what plant traits are most important in the mixtures, and additional research in this direction is required.

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