Making the results of evolutionary breeding in spring wheat functional for farmers, using a chain perspective

Experiences 2014-2016, final report

Edwin Nuijten
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Summary

Up to today little effort is being made in breeding spring wheat for organic farming. To diminish this dependence an alternative breeding approach is evolutionary breeding. It is a cost effective breeding method aimed to create high levels of genetic diversity in population varieties. This diversity results in improved yield stability, important for organic conditions. Farmers can propagate the seed of populations developed by breeders. Simple forms of farmer selection (through selection on seed size and plant length) may support the development of the populations in the right direction. However, the effects of such selection on baking quality is not yet clear. In this project we have studied two aspects important for successful cultivation: 1) The effect of farmer selection on the field performance of populations and their baking quality, and 2) understanding key elements for successful implementation in the whole production chain. In the Netherlands and in Germany both forms of selection were conducted for two seasons. In the third year all selection strategies were compared in a single trial.

The analysis of the trial data showed that the clearest selection effects are visible with the selection on seed size. This selection has clearest effects on morphological traits (percentage of bearded ears and brown ears). No clear selection effects were observed for yield and baking quality. As may be expected, a higher thousand kernel weight was observed for the selection on seed size. For one population selection on seed size resulted in taller plants, but not for the other population. No clear negative associated effects were observed for lodging tolerance. It means that farmers may need to be more careful with multiplication of certain populations. Selection on plant length did not have clear effects which can be explained by the low selection pressure.

On the one hand it means improvement of the population through simple selection measures does not happen quickly. On the other hand, it also suggests that the populations are robust in yield, agronomic traits and baking quality parameters. It means adaptation to local conditions does not happen that fast. It means that multiplication over three cycles did not have a positive effect on yield (Hypothesis one). For the baking quality parameters, the multiplication over three cycles did not have clear negative effects (Hypothesis two). Hence, we may conclude that mild forms of farmer selection will not have a positive impact on plant height and yield, and will not have any negative impact on baking quality traits compared to the original population (Hypothesis three). Detailed analysis suggests that the baking quality of the populations becomes more stable. Hence, the baking quality of the developed populations became more stable and is acceptable to bakeries (Hypothesis four). In conclusion, mild forms of farmer selection will not have negative impact on plant height and yield, and may have stabilising impact on baking quality traits (all hypotheses).
The selection effects on morphological traits were clearly visible. This helps explain that the morphology of the populations in farmer fields have changed in two years’ time. The farm machinery for harvesting is built differently compared to the machinery for harvesting trial plots. Depending on weed pressure, farmers may need to clean their harvests in different ways, in that way unconsciously changing the morphology of the populations they grow. Another trial for comparison showed that these farmer grown populations can change morphologically quite quickly in a few seasons. Field measurements and baking tests showed that the populations did not clearly change in agronomic performance and baking quality.

With farmers and bakers discussions were held on the potential of populations. The following lessons were learnt on cultivation and baking quality:

- The populations appeared to have advantages on sandy soils because of their taller plant height and hence better weed suppression. On clayey soils in the western part of the Netherlands, where yellow rust pressure is very high, their resistance to yellow rust is not good enough.
- A short chain is successful if farmers and bakers share ideas on agriculture. For farmers it is important to work with bakeries who want to work with wheat that can vary in quality and are able to adapt the baking process to the quality of the wheat, as differences in baking quality between the produce of different fields can occur.

The concept of populations fits well to farmers working on sandy soils in the eastern part of the Netherlands. They have different variety requirements compared to the large scale farmers working on the ‘optimal’ clayey soils in the western part of the Netherlands. In the meanwhile various organic breeders are developing new populations with better disease resistance and yield, and at the same time good baking quality compared to the first developed populations. Such populations are needed for further upscaling in the western part of the Netherlands. The results of this research were shared in the EU-wide research program COBRA.
1 Introduction

Up to today little effort is made in breeding spring wheat for organic farming. Unlike for winter wheat, organic farmers cannot rely on a constant development of new spring wheat varieties suited for organic farming. For example, in the Netherlands farmers rely on one spring wheat variety, Lavett, for almost 20 years now. It is not clear whether and if so when another good variety will be identified. One reason is that conventional bred spring wheat varieties do not have a good baking quality when grown under organic conditions (Wolfe et al., 2008), particularly in the Netherlands (Osman et al., 2008). Other traits rare in conventional bred varieties but important for organic farming are traits like rapid early growth and long straw, which are important to suppress weeds (Baresel, 2006; Lammerts van Bueren et al., 2010).

To diminish this dependence an alternative breeding approach is evolutionary breeding. Currently this approach is being exploited in organic agriculture in many countries for winter wheat (Löschenerberger et al. 2008, Wolfe et al. 2008), but not yet for spring wheat which is becoming more important for Northern European countries such as the Netherlands. Evolutionary breeding is particularly of interest for organic agriculture (Philips and Wolfe, 2005; Murphy et al., 2005). Evolutionary breeding consists of creating highly diverse populations (Composite Cross populations) and multiplying these in bulk for several generations. In the first generations various selection methods can be used by a breeder. In the following generations at the farm, the population will adapt further to the farm conditions. This approach is a simple and cost effective breeding method. Especially for low input conditions improved yield stability has been observed with composite cross populations (Nuijt and Lammerts van Bueren, 2013; Phillips and Wolfe, 2005; Danquah and Barrett, 2002). The improved yield stability is the result of more genetic diversity within a variety. More genetic diversity within varieties results in more buffering capacity and it also makes a crop more resilient against stresses (Wolfe, 1985; Finckh, 2008). As such, evolutionary breeding is also beneficial in maintaining crop genetic diversity in general.

Once a composite cross population is developed it may become more adapted to the farm conditions in the consecutive generations. As such it can also co-evolve with new climatic conditions and adapt itself to new growing conditions on a farm. A general concern, however, is that environmental selection may need to be complemented by simple forms of farmer selection to prevent the population to increase in height (Wolfe et al., 2013). Which form of selection is most suitable for that is not yet clear. For example, roguing non desired plant types, in particular plant that are too tall, may be a strategy to prevent a variety from becoming too tall. Another option may be selection for seed size. In that way seeds affected by fungi, in particular Fusarium, are removed, maintaining a healthy population of seed.
Another concern is that the effect of natural selection and farmer selection on baking quality is not yet clear. Baking quality traits are not directly correlated to fitness and therefore evolutionary breeding is not expected to improve bread making properties of wheat (Dawson et al., 2008). Breeders have the tools to combine selection for baking quality with natural selection (Murphy et al. 2005). Whether natural selection in farmer fields may have a stabilising impact on baking quality is also not yet clear. For a successful implementation of composite cross populations, the other chain players, in particular bakeries, need to be sure that the quality does not decrease over consecutive generations. In addition, particular bakeries prefer a relatively constant quality (Nuijten et al., 2011). Unlike for yield, a higher level of diversity within varieties seems not to have a stabilising effect on baking quality (Nuijten and Lammerts van Bueren, 2013). Whether continuous cultivation over several seasons will have a stabilising effect on baking quality is not yet clear.

In the proposed research we will study two aspects important for successful cultivation of composite cross populations of spring wheat. The yield and baking quality of a number of populations of spring wheat developed by Getreidezüchtungsforschung Dottenfelderhof (GD) and tested in the Netherlands was good (Nuijten and Lammerts van Bueren, 2013). The first aspect to study is the effect of different forms of farmer selection on the performance of varieties in the field and on baking quality. The second issue is a better understanding of the aspects to make successful implementation in the whole production chain feasible.

The results of this research will be shared in particular with the EU-wide research program COBRA (Coordinating organic plant breeding activities for diversity), in which 20 countries are involved, that focuses on improving population breeding for cereals and legumes. The results will be particular useful to identify and remove structural barriers in the production chain to develop organic plant breeding and seed production across Europe.

The aim and hypotheses of this research

The focus of this research is to study two aspects important for successful cultivation of composite cross populations of spring wheat:

- The effect of different forms of farmer selection on the performance of population varieties in the field (in particular plant height and yield) and on baking quality.
- A better understanding of the aspects to make successful implementation in the whole production chain feasible.

The hypotheses that will be tested are:

1. CCPs that have been multiplied three cycles at an organic farming site will have improved yield in comparison to the original population.
2. Baking quality traits (protein content, SDS-sedimentation, wet gluten content, gluten index, Hagberg Falling Number and loaf volume) of CCPs that have been multiplied for three generations will be similar to the original population.

3. Mild forms of farmer selection will have positive impact on plant height and yield, and will not have any negative impact on baking quality traits (see hypothesis 2) compared to the original population.

4. The baking quality of the developed CCPs will become more stable over the three generations, and are acceptable to bakeries.
2 Material and Methods

Research period
January 1, 2014 – December 31, 2016

Populations and standard varieties
Two populations to be used for this study are developed by Getreidezüchtungsforschung Dottenfelderhof in Germany and adapted to Dutch conditions through cultivation over three seasons from 2011 to 2013. These two populations are the populations HS 2-8 and HS 4-9. The CCPs are developed through an evolutionary breeding scheme, in which F4 populations with outstanding performance for baking quality traits (wet gluten content, gluten index, SDS sedimentation value and Hagberg Falling Number) and agronomic traits (plant health, vigour, yield) are mixed into new populations. For comparison, also two pure line spring wheat varieties (Lavett and Heliaro) and a new population (HS 1-12) were included.

Research sites
The two CCPs and pure line spring varieties were sown at two locations:
Site 1: organic farm with sandy soils in the Netherlands
Site 2: organic (bio-dynamic) breeding nursery of GD in Germany. This site served as a reference to follow the developments at Site 1 in 2014 and 2015.

Research set-up
Per year, the set-up of the trials differed according to the needs (See Figure 1):

2014 and 2015
In 2014 and 2015, trials set up and methods were similar. Of two CCPs, HS-2-8 and HS-4-9, two versions were cultivated at Site 1 and Site 2: the original CCPs that have undergone natural selection in the past three seasons in the Netherlands (population names with ‘O’ added), and the breeder selections in Germany (population names with ‘D’ and ‘NL’ added). Dutch farmers had a slight preference for the natural selections, because of better tillering, and the breeder had a preference for the improved breeder selection for the same reason, lower tillering. Alongside, two reference varieties, Lavett and Heliaro, were cultivated. The variety Lavett is the common spring wheat variety for organic cultivation in the Netherlands. The variety Heliaro was developed by Dottenfelderhof and adapted to organic farming in Germany.

At site 1 four selection strategies were followed: natural selection, selection for plant height, selection for seed size and selection through reduced sowing density. At site 2 two selection strategies were followed: natural selection and combined selection for plant height and seed size in the breeder selections. Before sowing, selection for seed size was conducted at both sites: at Site 1 on part of the natural selection, at Site 2 on part of the breeder selections.
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(population names with ‘S’ added). With selection for seed size, about 30% of the seed was remained for sowing. Shortly before harvest at both sites the long ears have been removed; at Site 1 in the natural selection, at Site 2 in the breeder selections (population names with ‘L’ added). This was done by removing ears of plants that were clearly higher: at least twice ear lengths higher compared to the average plants. At site 1, selection through reduced sowing density (50% of the normal density) was done in separate plots to see whether reduced plant density can increase tillering ability.

At both locations, yield, agronomic parameters (plant height and disease resistance), and baking quality parameters (protein content, SDS-sedimentation, wet gluten content, gluten index, Hagberg Falling Number and loaf volume) of the two CCPs and the two varieties were measured.

2016
All selections were sown at Site 1: The four Dutch selections of the two CCPs of 2015, stored seeds of the original CCPs sown in 2014, the three German selections of the CCPs grown at Site 2 and the two standard varieties. The parameters yield, agronomic parameters (thousand kernel weight, lodging tolerance, tillering, plant height and disease resistance), and baking quality parameters (protein content, SDS-sedimentation, wet gluten content, gluten index, Hagberg Falling Number and loaf volume) of the two CCPs and the two varieties were measured.

Trial Lay-Out and harvest
At site 1, the trial was sown in a farmer field in a randomized block design with four replications. At site 2 the trials were sown in a randomized block design with three replications. Sowing and harvesting were conducted with a plot sowing and harvesting machines at both locations.
Baking trials

Baking trials and bread evaluation were carried out in close collaboration with a commercial organic bakers. The baking recipe included whole meal, water, sour dough, yeast and salt, without any further additions of bread improvers.

Overall evaluation of the yield and baking quality

Analyses of yield, agronomic traits and baking quality parameters are conducted with ANOVA. In addition, morphological traits were compared. For the analysis, additional data were used from another project (Divers en Dichtbij) for the year 2016. In this way two Dutch locations were available for 2016, site 1 being called Menkveld, and the additional location being called Vos (both after the farmer’s names).

Every year, with a group of farmers and bakers, the results were discussed. With these stakeholders several meetings a year were held to discuss obstacles and opportunities to develop regional production chains, and in particular how to deal with these obstacles.
3 Results

General comparison of populations and varieties

Over the period 2014-2016, the variety Lavett had the highest yield and at the same the lowest results for the baking quality parameters in the Netherlands (Table 1). The variety Heliaro had yield and results for the baking quality similar to the populations. These results are somewhat different from the period 2011-2013 when the yield of the populations and varieties was quite similar.

In Germany the populations had better yield than the varieties in 2014 and 2015 (Table 2a, 2b), and similar yields in the period 2011-2013 (report 2010-89). The yield difference in Germany in 2014 and 2015 may be the result of selection on seed size. So, likely, the populations and varieties had similar yields in 2014 and 2015.

At both sites, the disease yellow rust was abundant in 2014 and 2015. It is often said that disease susceptibility affects yield, but this does not show clearly in the yield data. Of the populations, the new population HS 1-12 had the highest yields, and also best scores for yellow rust resistance in both countries (table 2). The yield level of this new population (HS 1-12) was among the highest measured in both sites.

Effects of selection strategies: results in 2016

Yield

When comparing the results for the selections done in the Netherlands, the various selection strategies seem not to have a clear effect on yield. A comparison of the selections done in the Netherlands and Germany suggests a higher yield for the German selections compared to the Dutch selections. This may be caused by the fact that the German selections were selected for larger seed size, whereas in the Netherlands only one selection was selected for larger seed size. A comparison of only the selections for larger seed size from both the Netherlands and Germany shows no significant difference. This suggests that selection on seed size can have a positive effect on yield.

Baking quality parameters

Whether the selection strategies have an effect on baking quality parameters is not clear. For sedimentation, significant differences were found that suggest opposite selection effects: the original population of HS 2-8 had a significant higher value for sedimentation, whereas the original population of HS 4-9 had a significant lower value for sedimentation. Among the selection strategies no significant differences for sedimentation were observed. The selections of HS 4-9 show significant differences for falling number, whereas the selec-
tions of HS 2-8 do not show any differences. The difference in wet gluten between the original populations of HS 2-8 and HS 4-9 was not as clear as in the period 2011-2013. Possibly this may be the result of natural selection.

**Agronomic traits**

Of the agronomic traits, an effect of selection was observed for thousand kernel weight (TKW). The selections for seed size have higher TKW than the original population and the other selections. For population HS 2-8, selection on seed size seems also to have an increasing effect on plant height. This increase in plant height was not visible for population HS 4-9.

The German populations seem to have better Septoria resistance, which seems also related to selection for larger seed size: when comparing only the Dutch and German selections for seed size, no difference is found. This is different for yellow rust resistance. The German selections on seed size show better resistance compared to the Dutch selections on seed size. This is likely the result of earlier selection done by the breeder on the German selections in the period 2011 to 2013. In 2013 a positive effect was shown of the selection by the breeder (report 2010-89).

**Effects on adaptability**

Results from the trials in Germany and the Netherlands in 2014 and 2015 provide information about the extent of improvement in adaptability of the populations in reference to two varieties. A good comparison with the period 2011-2013 is difficult as one of the reference varieties (Thasos) has been replaced with another variety (Heliaro) as the yellow rust resistance of the variety Thasos has broken down. The results show that that the varieties seem more stable for baking parameters and that the populations seem more stable for field traits. In terms of yield, the populations and varieties appeared to have similar levels of adaptability.

Analysis of the combined data of 2014 and 2015 shows that populations and varieties had no significant interactions for the traits yield, wet gluten, Hagberg falling number and plant length (Tables 3a and 3b). Both populations and varieties show significant interactions for glutenindex. For yellow rust, the varieties show a significant country x accession interaction. For SDS sedimentation, the populations show a significant year x accession interaction.

The ANOVA results for 2014 and 2015 separately (Tables 4 and 5 respectively) confirm that the pure line varieties are more adaptable for the baking quality parameters (Hagberg falling number in 2014 and percentage wet gluten in 2015) than the populations. This was also observed in the period 2011-2013. Interestingly, such results did not clearly show in results of the baking tests.
In 2014 and 2015, a clear Genotype x Environment interaction was visible for yellow rust incidence for the varieties (Tables 4c and 5c). This interaction showed the same pattern in both years: The variety Lavett was less affected in Germany and the variety Heliaro was less affected in the Netherlands. However, on average, the varieties tend to have better yellow rust resistance than the tested populations (Table 1). So, the adaptability in yellow rust resistance of the populations combines with higher levels of yellow rust incidence, which is a negative result.

For 2016, an analysis is made with data from the same populations in another trial in the Netherlands, confirming that the populations are adaptable in agronomic traits, but not for the baking quality parameters (Table 7).

**Effects on stability**

Comparing the year effects in one location in the Netherlands, the varieties have more significant p-values for the accession x year interaction terms than the populations (table 6). However, table 3 suggests there are no clear differences in accession x year interaction between varieties and populations. Part of this contradiction may be explained that the trait lodging tolerance cannot be included in the analysis of which the results are shown in table 3, whereas it is included in the analysis of which the results are shown in table 6. This analysis confirms that populations seem more stable in field traits than varieties.

**Baking tests and farmers views**

In the baking tests there seemed no clear relationship between baking quality parameters and the baking tests. Differences in baking test results were clear between farms, but differences were not that clear between populations, or between the populations and the varieties Lavett and Heliaro.

From discussions with farmers it emerged that it is important to have good cooperation with a baker to be able to sell the produce. In 2014, most produce was sold to a bio-dynamic baker in Germany. A large Dutch baker also showed interest in working with the populations, and it seems plausible he will continue working with populations in the future.

In 2014 and 2015, yellow rust pressure was high in the populations compared to the variety Lavett at the farmer fields close to the North See. On the farms more inland the yellow rust pressure was lower and the difference with the varieties Lavett was smaller. One farmer in the Southwest, close to the North Sea stopped cultivating the populations because of lack of yellow rust resistance and because of lack of market.
There is the idea that plants developed from larger seeds have better seedling viability and hence better overall tolerance against yellow rust. None of the farmers had selected for seed size, however, because of fear of narrowing the populations.

**Morphological traits**

Whereas the effects of selection on agronomic traits and baking quality parameters were relatively small, clear effects were visible for the morphological traits such as greenness (waxiness) and percentage of bearded ears (Table 8). Selection effects on greenness were visible for both populations, and for selections in both the Netherlands and Germany. In the Netherlands, the original populations have the highest percentage of greenness, whereas the selections on seed size have the lowest percentage. A similar effect is visible for population HS 4-9 in Germany, but the opposite effect for population HS 2-8 of which the selections on seed size have the highest percentage of greenness.

Selection effects for percentage of bearded ears was only visible for population HS 2-8, which is due to the fact that population HS 4-9 has very low percentages of bearded ears (on average lower than 1 percent). For population HS 2-8 selection on seed size resulted on clearly higher percentages of bearded ears in the Netherlands, but not in Germany.
4 Discussion

Selection effects on agronomic traits and baking quality

Overall, the analysis based on the trial data from 2016 show that the clearest selection effects are visible with the selection on seed size. This selection has clearest effects on morphological traits. As may be expected, also a higher TKW was observed for selection on seed size. For population HS 2-8 selection on seed size resulted in taller plants, but not for population HS 4-9. No clear negative associated effects were observed for lodging tolerance. It means that farmers may need to be more careful with multiplication of the population HS 2-8 than with population HS 4-9. On the other hand, as daily practice most farmers will not practice such intense selection on seed size. No clear selection effects were observed for yield, baking quality parameters and other agronomic traits.

The other selection strategies did not have clear effects compared to the original population that was stored at the start of the project and sown in the last year. The natural selections in both the Netherlands and Germany did not clearly differ from the original population. Selection on plant length in the Netherlands did not have clear effects which can be explained by the low selection pressure. Improving the population through sowing in low density also did not have any clear effects.

On the one hand it means improvement of the population through simple selection measures does not happen quickly. On the other hand, it also suggests that the populations are robust in yield, agronomic traits and baking quality parameters. It means adaptation to local conditions does not happen that fast. It means that multiplication over three cycles did not have a positive effect on yield (Hypothesis one). Except for the baking quality parameter Zeleny sedimentation, the multiplication over three cycles did not have clear negative effects (Hypothesis two). In regards to Zeleny sedimentation, population HS 2-8 showed a slight decrease and population HS 4-9 showed a slight increase compared to the original population. These changes were significant. Remarkable is that the selections of populations HS 2-8 and HS 4-9 have similar values. In the case of population HS 2-8 the question is whether the population is gradually developing towards a relatively stable status quo in terms of protein quality. Hence, we may conclude that mild forms of farmer selection will not have positive impact on plant height and yield, and will not have any negative impact on baking quality traits compared to the original population (Hypothesis three). However, severe forms of selection can have negative impact on plant height without having positive impact on yield. Hence, it is best that farmers apply mild forms of selection rather than severe forms of selection. A comparison of the ANOVA results for 2014 and 2015 suggests that the baking quality of the populations becomes more stable. A comparison of the baking quality parameters over three years based on the location Menkveld seems to confirms that baking quality can become more stable. However, these results may also be the result of field conditions. Based on this information we need to be careful to say that the baking quality of the developed CCPs will become more stable over the three generations, and that they are acceptable to bakeries (Hypothesis four).
In conclusion, mild forms of farmer selection will not have negative impact on plant height and yield, and may have stabilising impact on baking quality traits (all hypotheses). In this experiment, we have compared two trial sites which have a relatively low disease pressure in many years. Further insights from another experiment in a location with severe disease pressure, e.g., yellow rust in particular (the location Vos), will provide more information on the effect of selection through disease pressure on baking quality.

**Selection effects on morphology**

The results also show that even though selection does not seem to have much effect on agronomic traits and on baking quality parameters, the effects on morphological traits are clearly visible. The clearest effect is visible for selection on seed size on the percentage of bearded ears in the case of population HS 2-8 in the Netherlands. This may also help explain that the morphology of the populations in farmer fields have changed in two years’ time. The farm machinery for harvesting and cleaning is built differently compared to the machinery for harvesting and cleaning trial plots. Depending on weed pressure, farmers may need to clean their harvests in different ways, in that way unconsciously changing the morphology of the populations they grow. A trial for comparison set up by the Dutch regulation authorities (NAK and NAKtuinbouw) showed that these farmer grown populations can change morphologically in a few seasons quite quickly. Field measurements and baking tests showed that the populations did not clearly change in agronomic performance and baking quality. With NAK and NAKtuinbouw, we will continue designing an example of the content of article 14.3 about heterogeneous materials of the EU seed law. This will also be based on the input and experiences of the farmers.

**Lessons learned**

With farmers and bakers discussions were held on the potential of populations. The following lessons were learnt on cultivation and baking quality (Nuijten 2016):

- The populations appeared to have advantages on sandy soils because of their taller plant height and hence better weed suppression. On clayey soils in the western part of the Netherlands, where yellow rust pressure is very high, their resistance to yellow rust is not good enough.
- A short chain is successful if farmers and bakers share ideas and vision on agriculture. For example, if scores for baking quality are considered relative. In 2014 there appeared to be little relationship between protein content and baking quality (Figure 1).
- For farmers it is important to work with bakeries who want to work with wheat that can vary in quality. A baker needs to be prepared to adapt the baking process to the quality of the wheat, as differences in baking quality between the produce of different fields can occur.

Meetings with regulation authorities (NAK and NAKtuinbouw) resulted in the following lessons learned on regulations (Nuijten 2016):

- Rethinking is required by authorities: they are used to work with uniform material. Working with heterogeneous material requires a different approach.
• A different approach is needed to describe CCPs: can agronomic and baking quality traits be used instead of morphological traits? However, in farmers’ fields the differences found between the two CCPs appeared to be relatively small. Morphological traits appeared to be rather unstable.

• Guidelines are developed to reduce the risk of mixture of the CCPs. In case of accidental mixture farmers can buy seed from fellow farmers, for which special regulations for seed replacement apply. For that adjusted rules for seed production apply. It is also useful if the seed testing authority has samples of the CCPs available for comparison.

Future perspectives for CCP’s

It seems that for now the concept of CCPs fits well to farmers working on sandy soils in the eastern part of the Netherlands. They have different variety requirements compared to the large scale farmers working on the ‘optimal’ clayey soils in the western part of the Netherlands. In the meanwhile various organic breeders are developing new populations. It seems the population HS 1-12 has better disease resistance, better yield, and at the same time good baking quality compared to the first developed populations. Another question is whether selection under severe disease pressure results in improved population with a good balance in disease pressure, yield and baking quality. Such populations are needed for further upscaling in the western part of the Netherlands. Also, measures to distinguish populations need to be further studied.
References


Appendix – Tables & Figures

Table 1: Yield, field performance and baking quality parameters at the Dutch trial site from 2014 to 2016 (analysed with ANOVA).

Table 2a: Yield, field performance and baking quality parameters at the Dutch and German trial sites in 2014 (analysed with ANOVA).

Table 2b: Yield, field performance and baking quality parameters at the Dutch and German trial sites in 2015 (analysed with ANOVA).

Table 2c: Yield, field performance and baking quality parameters of all Dutch and German accessions grown at the Dutch trial site in 2016 (analysed with ANOVA).

Table 3a: p-values for the populations for yield, field performance and the baking quality parameters based on two sites in 2014 and 2015 (analysed with ANOVA).

Table 3b: p-values for the varieties for yield, field performance and the baking quality parameters based on two sites in 2014 and 2015 (analysed with ANOVA).

Table 3c: Significance levels for yield, field performance and the baking quality parameters in 2014 based on all accessions in two locations in the Netherlands and Germany (analysed with ANOVA).

Table 4a: Significance levels for yield, field performance and the baking quality parameters in 2014 based on varieties in two locations in the Netherlands and Germany (analysed with ANOVA).

Table 4b: Significance levels for yield, field performance and the baking quality parameters in 2014 based on populations in two locations in the Netherlands and Germany (analysed with ANOVA).

Table 4c: Significance levels for yield, field performance and the baking quality parameters in 2014 based on varieties in two locations in the Netherlands and Germany (analysed with ANOVA).

Table 5a: Significance levels for all accessions for yield, field performance and the baking quality parameters in 2015 in two locations in the Netherlands and Germany (analysed with ANOVA).

Table 5b: Significance levels for the populations for yield, field performance and the baking quality parameters in 2015 in two locations in the Netherlands and Germany (analysed with ANOVA).

Table 5c: Significance levels for the varieties for yield, field performance and the baking quality parameters in 2015 in two locations in the Netherlands and Germany (analysed with ANOVA).

Table 6a: Significance levels for all accessions for yield, field performance and the baking quality parameters in the main location in the Netherlands over three years, from 2014 to 2016 (analysed with ANOVA).

Table 6b: Significance levels for the populations for yield, field performance and the baking quality parameters in the main location in the Netherlands over three years, from 2014 to 2016 (analysed with ANOVA).

Table 6c: Significance levels for the varieties for yield, field performance and the baking quality parameters in the main location in the Netherlands over three years, from 2014 to 2016 (analysed with ANOVA).

Table 7a: Averages for three populations for yield, baking quality parameters and agronomic traits in 2016 in two locations in the Netherlands.

Table 7b: Significance levels for the populations for yield, field performance and the baking quality parameters in 2016 based two sites in the Netherlands (analysed with ANOVA).

Table 8: Averages for the morphological traits greenness of the stalk, brown ears and bearded ears of two populations and the various selection types conducted in the Netherlands (NL) and Germany (D) in two locations (Menkveld and Vos) in 2016.

Figure 1: Slices of the baked breads of the populations with the pure line varieties Lavett (nr 5) and Heliaro (nr 10) for comparison. Protein contents are printed on top.
<table>
<thead>
<tr>
<th>accession</th>
<th>year</th>
<th>yield (ton/ha)</th>
<th>Wet gluten (%)</th>
<th>Gluten index (%)</th>
<th>SDS sedimentation (ml)</th>
<th>Hagberg falling number (s)</th>
<th>Plant length (cm)</th>
<th>Lodging tolerance (1-9)</th>
<th>Yellow rust (1-9)</th>
<th>TKW (in gr)</th>
<th># ears / m²</th>
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<td>2014</td>
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<td>18.7</td>
<td>88.5</td>
<td>48.3</td>
<td>372.2</td>
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<td>7.8</td>
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<td>523.4</td>
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Table 2a: Yield, field performance and baking quality parameters at the Dutch and German trial sites in 2014 (analysed with ANOVA).

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* O = Original population, S = selection for seed size, L = selection for Plant Length; + LSD = Least significant difference
### Table 2b: Yield, field performance and baking quality parameters at the Dutch and German trial sites in 2015 (analysed with ANOVA).

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<th>Gluten index (%)</th>
<th>SDS sedimentation (ml)</th>
<th>Hagberg falling number [s]</th>
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* O= Original population, S = selection for seed size, L = selection for plant length; * LSD = Least Significant Difference
Table 2c: Yield, field performance and baking quality parameters of all Dutch and German accessions grown at the Dutch trial site in 2016 (analysed with ANOVA).

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<th>Gluten index (%)</th>
<th>SDS sedimentation (ml)</th>
<th>Hagberg falling number [s]</th>
<th>Plant length (cm)</th>
<th>Lodging tolerance (1-9)</th>
<th># ears / m²</th>
<th>Yellow rust resistance (1-9)</th>
<th>Septoria resistance (1-9)</th>
<th>Thousand kernel weight (in gr)</th>
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* O= Original population, S = selection for seed size, L = selection for plant length; D = selection through low sowing density; * LSD = Least Significant Difference;
Table 3a: p-values for the populations for yield, field performance and the baking quality parameters based on two sites in 2014 and 2015 (analysed with ANOVA).

<table>
<thead>
<tr>
<th>Term</th>
<th>yield (ton/ha)</th>
<th>Wet gluten (%)</th>
<th>Gluten index (%)</th>
<th>SDS sedimentation (ml)</th>
<th>Hagberg falling number (s)</th>
<th>Plant length (cm)</th>
<th>Yellow rust (1-9)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Year</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
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<td>Year x accession</td>
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<td>0.709</td>
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<td>0.515</td>
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<tr>
<td>Year x country x accession</td>
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<td>0.748</td>
<td>0.209</td>
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Table 3b: p-values for the varieties for yield, field performance and the baking quality parameters based on two sites in 2014 and 2015 (analysed with ANOVA).

<table>
<thead>
<tr>
<th>Term</th>
<th>yield (ton/ha)</th>
<th>Wet gluten (%)</th>
<th>Gluten index (%)</th>
<th>SDS sedimentation (ml)</th>
<th>Hagberg falling number (s)</th>
<th>Plant length (cm)</th>
<th>Yellow rust (1-9)</th>
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<tr>
<td>Year</td>
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<td>&lt;0.001</td>
<td>&lt;0.001</td>
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<td>&lt;0.001</td>
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<td>&lt;0.001</td>
<td>0.544</td>
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<td>&lt;0.001</td>
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<tr>
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<td>&lt;0.001</td>
<td>0.340</td>
<td>0.089</td>
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Table 4a: Significance levels for yield, field performance and the baking quality parameters in 2014 based on all accessions in two locations in the Netherlands and Germany (analysed with ANOVA).

<table>
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<td>yield (ton/ha)</td>
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<td>Plant length (cm)</td>
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<td>Thousand kernel weight (gram)</td>
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Table 4b: Significance levels for yield, field performance and the baking quality parameters in 2014 based on populations in two locations in the Netherlands and Germany (analysed with ANOVA).

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<td>p-value</td>
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Table 4c: Significance levels for yield, field performance and the baking quality parameters in 2014 based on varieties in two locations in the Netherlands and Germany (analysed with ANOVA).

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Table 5a: Significance levels for all accessions for yield, field performance and the baking quality parameters in 2015 in two locations in the Netherlands and Germany (analysed with ANOVA).

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<td>F p-value</td>
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<td>&lt;0.001</td>
<td>45.75</td>
</tr>
<tr>
<td># ears / m2</td>
<td>27.8</td>
<td>&lt;0.001</td>
<td>290.63</td>
</tr>
</tbody>
</table>

Table 5b: Significance levels for the populations for yield, field performance and the baking quality parameters in 2015 in two locations in the Netherlands and Germany (analysed with ANOVA).

<table>
<thead>
<tr>
<th>Trait</th>
<th>Accession</th>
<th>Country</th>
<th>accession x country</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>F p-value</td>
<td>F p-value</td>
</tr>
<tr>
<td>yield (ton/ha)</td>
<td>4.0</td>
<td>&lt;0.001</td>
<td>22.1</td>
</tr>
<tr>
<td>Wet gluten (%)</td>
<td>7.1</td>
<td>&lt;0.001</td>
<td>49.1</td>
</tr>
<tr>
<td>Gluten index (%)</td>
<td>12.3</td>
<td>&lt;0.001</td>
<td>11.1</td>
</tr>
<tr>
<td>SDS sedimentation (ml)</td>
<td>4.0</td>
<td>&lt;0.001</td>
<td>15.7</td>
</tr>
<tr>
<td>Hagberg falling number (s)</td>
<td>5.6</td>
<td>&lt;0.001</td>
<td>17.7</td>
</tr>
<tr>
<td>Plant length (cm)</td>
<td>5.8</td>
<td>&lt;0.001</td>
<td>37.2</td>
</tr>
<tr>
<td>Yellow rust (1-9)</td>
<td>6.5</td>
<td>&lt;0.001</td>
<td>24.5</td>
</tr>
<tr>
<td># ears / m2</td>
<td>28.7</td>
<td>&lt;0.001</td>
<td>165.5</td>
</tr>
</tbody>
</table>

Table 5c: Significance levels for the varieties for yield, field performance and the baking quality parameters in 2015 in two locations in the Netherlands and Germany (analysed with ANOVA).

<table>
<thead>
<tr>
<th>Trait</th>
<th>Accession</th>
<th>Country</th>
<th>accession x country</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>F p-value</td>
<td>F p-value</td>
</tr>
<tr>
<td>yield (ton/ha)</td>
<td>2.2</td>
<td>0.168</td>
<td>2.3</td>
</tr>
<tr>
<td>Wet gluten (%)</td>
<td>6.7</td>
<td>0.034</td>
<td>14.2</td>
</tr>
<tr>
<td>Gluten index (%)</td>
<td>84.0</td>
<td>&lt;0.001</td>
<td>75.4</td>
</tr>
<tr>
<td>SDS sedimentation (ml)</td>
<td>0.7</td>
<td>0.420</td>
<td>7.1</td>
</tr>
<tr>
<td>Hagberg falling number (s)</td>
<td>1.3</td>
<td>0.273</td>
<td>4.0</td>
</tr>
<tr>
<td>Plant length (cm)</td>
<td>2.8</td>
<td>0.123</td>
<td>19.8</td>
</tr>
<tr>
<td>Yellow rust (1-9)</td>
<td>0.0</td>
<td>0.898</td>
<td>34.7</td>
</tr>
<tr>
<td># ears / m2</td>
<td>0.6</td>
<td>0.468</td>
<td>215.4</td>
</tr>
</tbody>
</table>
Table 6a: Significance levels for all accessions for yield, field performance and the baking quality parameters in the main location in the Netherlands over three years, from 2014 to 2016 (analysed with ANOVA).

<table>
<thead>
<tr>
<th>Trait</th>
<th>accession</th>
<th>year</th>
<th>accession x year</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>F</td>
<td>p-value</td>
<td>F</td>
</tr>
<tr>
<td>yield (ton/ha)</td>
<td>4.5</td>
<td>0.004</td>
<td>24.7</td>
</tr>
<tr>
<td>Wet gluten (%)</td>
<td>5.1</td>
<td>0.002</td>
<td>148.2</td>
</tr>
<tr>
<td>Gluten index (%)</td>
<td>19.5</td>
<td>&lt;0.001</td>
<td>121.1</td>
</tr>
<tr>
<td>SDS sedimentation (ml)</td>
<td>4.3</td>
<td>0.005</td>
<td>411.2</td>
</tr>
<tr>
<td>Hagberg falling number (s)</td>
<td>18.0</td>
<td>&lt;0.001</td>
<td>338.0</td>
</tr>
<tr>
<td>Plant length (cm)</td>
<td>11.2</td>
<td>&lt;0.001</td>
<td>16.3</td>
</tr>
<tr>
<td>Lodging tolerance (1-9)</td>
<td>3.5</td>
<td>0.014</td>
<td>11.2</td>
</tr>
<tr>
<td>Yellow rust (1-9)</td>
<td>49.7</td>
<td>&lt;0.001</td>
<td>168.7</td>
</tr>
<tr>
<td>TKW (gram)</td>
<td>34.2</td>
<td>&lt;0.001</td>
<td>82.9</td>
</tr>
<tr>
<td># ears / m2</td>
<td>0.1</td>
<td>0.968</td>
<td>11.1</td>
</tr>
</tbody>
</table>

Table 6b: Significance levels for the populations for yield, field performance and the baking quality parameters in the main location in the Netherlands over three years, from 2014 to 2016 (analysed with ANOVA).

<table>
<thead>
<tr>
<th>Trait</th>
<th>accession</th>
<th>year</th>
<th>accession x year</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>F</td>
<td>p-value</td>
<td>F</td>
</tr>
<tr>
<td>yield (ton/ha)</td>
<td>3.9</td>
<td>0.032</td>
<td>13.8</td>
</tr>
<tr>
<td>Wet gluten (%)</td>
<td>1.0</td>
<td>0.397</td>
<td>95.6</td>
</tr>
<tr>
<td>Gluten index (%)</td>
<td>2.7</td>
<td>0.086</td>
<td>84.9</td>
</tr>
<tr>
<td>SDS sedimentation (ml)</td>
<td>1.0</td>
<td>0.381</td>
<td>279.3</td>
</tr>
<tr>
<td>Hagberg falling number (s)</td>
<td>17.7</td>
<td>&lt;0.001</td>
<td>262.0</td>
</tr>
<tr>
<td>Plant length (cm)</td>
<td>6.5</td>
<td>0.005</td>
<td>12.5</td>
</tr>
<tr>
<td>Lodging tolerance (1-9)</td>
<td>0.6</td>
<td>0.574</td>
<td>4.4</td>
</tr>
<tr>
<td>Yellow rust (1-9)</td>
<td>23.6</td>
<td>&lt;0.001</td>
<td>75.4</td>
</tr>
<tr>
<td>TKW (gram)</td>
<td>18.9</td>
<td>&lt;0.001</td>
<td>45.5</td>
</tr>
<tr>
<td># ears / m2</td>
<td>0.2</td>
<td>0.858</td>
<td>1.7</td>
</tr>
</tbody>
</table>

Table 6c: Significance levels for the varieties for yield, field performance and the baking quality parameters in the main location in the Netherlands over three years, from 2014 to 2016 (analysed with ANOVA).

<table>
<thead>
<tr>
<th>Trait</th>
<th>accession</th>
<th>year</th>
<th>accession x year</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>F</td>
<td>p-value</td>
<td>F</td>
</tr>
<tr>
<td>yield (ton/ha)</td>
<td>4.4</td>
<td>0.051</td>
<td>11.1</td>
</tr>
<tr>
<td>Wet gluten (%)</td>
<td>15.0</td>
<td>0.001</td>
<td>55.2</td>
</tr>
<tr>
<td>Gluten index (%)</td>
<td>35.6</td>
<td>&lt;0.001</td>
<td>37.2</td>
</tr>
<tr>
<td>SDS sedimentation (ml)</td>
<td>12.2</td>
<td>&lt;0.001</td>
<td>144.3</td>
</tr>
<tr>
<td>Hagberg falling number (s)</td>
<td>20.4</td>
<td>&lt;0.001</td>
<td>77.6</td>
</tr>
<tr>
<td>Plant length (cm)</td>
<td>23.2</td>
<td>&lt;0.001</td>
<td>4.4</td>
</tr>
<tr>
<td>Lodging tolerance (1-9)</td>
<td>22.5</td>
<td>&lt;0.001</td>
<td>19.2</td>
</tr>
<tr>
<td>Yellow rust (1-9)</td>
<td>15.6</td>
<td>&lt;0.001</td>
<td>108.6</td>
</tr>
<tr>
<td>TKW (gram)</td>
<td>35.2</td>
<td>&lt;0.001</td>
<td>45.4</td>
</tr>
<tr>
<td># ears / m2</td>
<td>0.2</td>
<td>0.627</td>
<td>20.1</td>
</tr>
</tbody>
</table>
### Table 7a: Averages for three populations for yield, baking quality parameters and agronomic traits in 2016 in two locations in the Netherlands.

<table>
<thead>
<tr>
<th>Accession</th>
<th>Locations</th>
<th>Menkveld and Vos</th>
<th>yield (ton/ha)</th>
<th>Wet gluten (%)</th>
<th>Gluten index (%)</th>
<th>SDS sedimentation (ml)</th>
<th>Hagberg falling number (s)</th>
<th>Yellow rust [1-9]</th>
<th>Septoria [1-9]</th>
<th>Plant length [cm]</th>
<th># aren</th>
</tr>
</thead>
<tbody>
<tr>
<td>HS 1-12</td>
<td>average</td>
<td>3.19</td>
<td>25.7</td>
<td>96.0</td>
<td>67.0</td>
<td>198</td>
<td>8.2</td>
<td>7.2</td>
<td>102.2</td>
<td>403</td>
<td></td>
</tr>
<tr>
<td>HS 2-8-O</td>
<td>average</td>
<td>3.00</td>
<td>24.4</td>
<td>93.9</td>
<td>65.3</td>
<td>224</td>
<td>7.8</td>
<td>6.7</td>
<td>98.9</td>
<td>389</td>
<td></td>
</tr>
<tr>
<td>HS 4-9-O</td>
<td>average</td>
<td>3.30</td>
<td>23.4</td>
<td>93.2</td>
<td>64.3</td>
<td>200</td>
<td>7.6</td>
<td>6.1</td>
<td>96.4</td>
<td>401</td>
<td></td>
</tr>
<tr>
<td>p-value</td>
<td>ns</td>
<td>&lt;0.001</td>
<td>ns</td>
<td>0.011</td>
<td>0.012</td>
<td>0.005</td>
<td>0.042</td>
<td>0.049</td>
<td>ns</td>
<td>ns</td>
<td></td>
</tr>
<tr>
<td>LSD</td>
<td>ns</td>
<td>1.63</td>
<td>ns</td>
<td>1.68</td>
<td>17.55</td>
<td>0.338</td>
<td>0.82</td>
<td>4.64</td>
<td>ns</td>
<td>ns</td>
<td></td>
</tr>
</tbody>
</table>

### Table 7b: Significance levels for the populations for yield, field performance and the baking quality parameters in 2016 based two sites in the Netherlands (analysed with ANOVA).

<table>
<thead>
<tr>
<th>Trait</th>
<th>Accession</th>
<th>Location</th>
<th>Accession x Location</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>F pr.</td>
<td>p-value</td>
<td>F pr.</td>
</tr>
<tr>
<td>Yield (ton/ha)</td>
<td>0.9</td>
<td>0.415</td>
<td>54.1</td>
</tr>
<tr>
<td>Wet gluten (%)</td>
<td>10.5</td>
<td>&lt;.001</td>
<td>467.6</td>
</tr>
<tr>
<td>Gluten index (%)</td>
<td>1.5</td>
<td>0.256</td>
<td>20.5</td>
</tr>
<tr>
<td>SDS sedimentation (ml)</td>
<td>6.2</td>
<td>0.011</td>
<td>284.4</td>
</tr>
<tr>
<td>Hagberg falling number (s)</td>
<td>6.1</td>
<td>0.012</td>
<td>3.7</td>
</tr>
<tr>
<td>Yellow rust [1-9]</td>
<td>7.6</td>
<td>0.005</td>
<td>137.4</td>
</tr>
<tr>
<td>Septoria [1-9]</td>
<td>4.0</td>
<td>0.042</td>
<td>0.5</td>
</tr>
<tr>
<td>Plant length [cm]</td>
<td>3.7</td>
<td>0.049</td>
<td>137.1</td>
</tr>
<tr>
<td># fertile tillers</td>
<td>0.2</td>
<td>0.843</td>
<td>180.7</td>
</tr>
</tbody>
</table>
Table 8: Averages for the morphological traits greenness of the stalk, brown ears and bearded ears of two populations and the various selection types conducted in the Netherlands (NL) and Germany (D) in two locations (Menkveld and Vos) in 2016.

<table>
<thead>
<tr>
<th>Accession and selection type</th>
<th>Site of selection</th>
<th>Stalk greenness (in %)</th>
<th>Brown ears (in %)</th>
<th>Bearded ears (in %)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Menkveld</td>
<td>Menkveld</td>
<td>Vos</td>
<td>Menkveld</td>
</tr>
<tr>
<td>HS 2-8-O 2013</td>
<td>1</td>
<td>39</td>
<td>11</td>
<td>19</td>
</tr>
<tr>
<td>HS 2-8-O</td>
<td>1</td>
<td>34</td>
<td>11</td>
<td>19</td>
</tr>
<tr>
<td>HS 2-8-D</td>
<td>1</td>
<td>29</td>
<td>8</td>
<td>20</td>
</tr>
<tr>
<td>HS 2-8-S</td>
<td>1</td>
<td>28</td>
<td>20</td>
<td>41</td>
</tr>
<tr>
<td>HS 2-8-L</td>
<td>1</td>
<td>35</td>
<td>13</td>
<td>16</td>
</tr>
<tr>
<td>HS 2-8-O</td>
<td>2</td>
<td>36</td>
<td>15</td>
<td>17</td>
</tr>
<tr>
<td>HS 2-8-D S+L</td>
<td>2</td>
<td>60</td>
<td>11</td>
<td>19</td>
</tr>
<tr>
<td>HS 2-8-NL S+L</td>
<td>2</td>
<td>56</td>
<td>19</td>
<td>21</td>
</tr>
<tr>
<td>HS 4-9-O 2013</td>
<td>1</td>
<td>34</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>HS 4-9-O</td>
<td>1</td>
<td>35</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>HS 4-9-D</td>
<td>1</td>
<td>30</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>HS 4-9-S</td>
<td>1</td>
<td>30</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>HS 4-9-L</td>
<td>1</td>
<td>28</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>HS 4-9-O</td>
<td>2</td>
<td>29</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>HS 4-9-D S+L</td>
<td>2</td>
<td>21</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>HS 4-9-NL S+L</td>
<td>2</td>
<td>23</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

* O = Original population, S = selection for seed size, L = selection for plant length; D = selection through low sowing density
Figure 1: Slices of the baked breads of the populations with the pure line varieties Lavett (nr 5) and He-liaro (nr 10) for comparison. Protein contents are printed on top.