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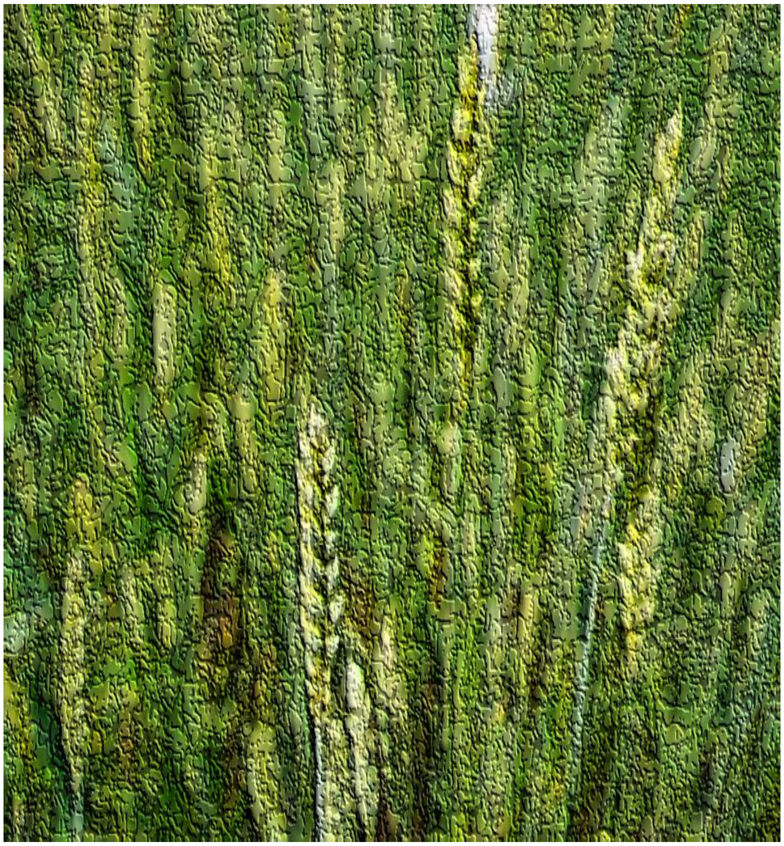
Composite cross populations (CCPs) of Winter wheat under low-input farming systems in the Netherlands: *G x E interaction, Adaptability and Stability of yield over years*



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Abbreviation

· BBCH: Biologische Bundesanstalt, Bundessortenamt and CHemical industry

· CCPs: Composite cross populations

· ΔG: Genetic advance

· G * E Interaction: Genotype x Environment interaction

· METs: Multi-environment trials

· M.S: Male sterility

· NUE: Nitrogen-use efficiency

· OPVs: Open pollinated varieties

· TKW: Thousand kernel weight

Glossary

- **Accessions:**
  A distinct, uniquely identifiable sample of seeds representing a cultivar, breeding line or a population, which is maintained in storage for conservation and use. In this paper, accessions include all CCPs and varieties

- **Adaptability:**
  A measure of the extent to which a species or ecosystem is able to adjust to environmental change

- **Assortative mating:**
  A form of nonrandom mating in which individuals select mates with a similar phenotype to themselves

- **Composite cross populations (CCPs):**
  Populations of segregating individuals; varietal mixtures. Instead of selecting promising individuals in each generation, the whole population is exposed to natural selection in each subsequent generation

- **Diminishing returns:**
  In economics, the law indicates that after a certain point, adding more people or machinery to the production process will not yield a proportionate greater amount of production

- **G * E interaction:**
  An inference drawn from the observation that the phenotypic expression of a given genotype varies when measured under different environmental conditions

- **Genetic advance:**
  The amount of increase in performance that is achieved after one generation has passed

- **Heritability:**
  A measure of the extent to which a characteristic in an organism is related to genetic, inherited factors relative to the mean of the population

$$h^2 = \frac{\text{genotypic var.}}{\text{phenotypic var.}}$$

$$= \frac{\text{genotypic var.}}{\text{genotypic var. + non genotypic var.}}$$

- **Multi-environment trials (METs):**
  Trials or experiments to assess the performance of genotypes across a range of locations in different environments

- **Selection pressure:**
  The extent to which organisms possessing a particular characteristic are either eliminated or favored by environmental demands

- **The response to selection (R):**
  The difference between the mean of the offspring from the selected parents and of the whole of the parental generation before selection

- **The selection differential (S):**
  The difference between the mean of the selected plants and of the base population

- **Variety**
  Commercial cultivars. In this paper, varieties indicate *Naturastar* and *Julius*

Acknowledgment

*Nanos gigantum humeris insidentes**

I admired ‘*George Washington Carver’* when I was in junior high. Through his life story, I decided to choose agronomy as a my major. Now, the more I keep studying my major, the more I cannot help but respect academic pioneers like *George Washington Carver or* someone whom I met today to discuss about academic subjects in campus. Through this field research, I realized that I have a long way to go to reach the same place as where they are standing. I thank Dr. *Edith Lammerts van Bueren* who gave me the opportunity to participate in this research. She provided me with a wonderful playground where I can play. I believe that I could finish this research, thanks to her warm supports. I also thank *Edwin Nuijten* who was my co-supervisor in this research. It was a great opportunity for me to visit wheat fields with him and learn about wheat growing from him. His field expertise is what I would like to have in the future. I also thank my parents who believe and support my dream. Now I can understand that their love cannot be described with anything. Finally, I thank Mr. Jeong who supported my dream financially. When he said that he wants to invest in my future, I gained more confidence in my decision of returning back to the campus. Someday, I also would like to be a person like him, supporting other people’s dreams. With many people who I appreciate, now, I would like to move forward to a new beginning. In this new beginning, I will not struggle to reach the place where our academic pioneers are standing. Instead, as Isaac Newton said; ‘*If I have seen further than others, it is by standing upon the shoulders of giant,*’ I will stand on the shoulders of them. I would like to follow their footprint in a humble way and learn from their academic achievements. Once again, I thank all academic giants who I met during my research. From time to time, a word from them gave me new ideas. Someday, I would like to be a person like them who can give various ideas to a new beginner through my academic achievements. Finally, through this research, I found out when I am on my happiest state. I was being the happiest person when I'm in a farm field. Therefore, in the future, when I read this paper again, I hope I will realize that this research was a new start in discovering my career path.

Jin.W.Kim

* *Standing on the shoulders of giants*

Abstract

In growing season 2015/2016, 9 different composite cross populations (CCPs) and two commercial varieties were evaluated in order to elucidate the evolutionary breeding in winter wheat through the analysis of genotype performance over year under low-input farming systems. All 11 accessions showed a germination rate of more than 90% but there was no significant difference among the 11 accessions. It indicates that all accessions have the same seed quality with respect to the vigor and that the difference of early seedling emergence among accessions results from the genotype variance of each accession. In the early growing season, no correlation was found between early seedling emergence and tillering in CCPs whereas two pure line varieties showed the positive correlation. Furthermore, compared to CCPs, the two varieties showed a lower reduced rate (%) of tillering, which indicates that most of the early tillerings became possible fertile tillers. It also indicates a low buffering capacity. Under high environmental variability, seedling emergence in a growing field might be the most vulnerable trait. Also, when environmental conditions are unpredictable, early tillering will be dramatically reduced and it will cause low fertile tillering if the tillering has a low buffering capacity. Therefore, this result suggests, for tillering, CCPs have more resilience and buffering capacity against environmental variability than the two varieties. Also, in this research, it is suggested that weed competitiveness on the basis of crop characteristics is not enough to fully explain weed suppression as there are various weed species, which show different physiological and morphological characteristics. Also, under evolutionary breeding, this research suggests that a different theory regarding the advantages of the distance between the flag leaf and spike because in this research, no relationship was found between the distance and the actual spike disease (%). For CCPs populations, plant height is highly heterogeneous. It means spores in longer plants can easily move to adjacent short plants and infect the spike. Therefore, the distance might not always be a crucial breeding goal in evolutionary breeding as there are too many variables to simply define the advantages of the long distance between leaf and spike under low-input farming systems. In the multiple regression model of both the plant height and flag leaf length, the coefficient (β) of the ground cover rate was much larger than other variables. Therefore, the ground cover rate can be considered as the most important factor that affects the plant height and flag leaf in CCPs. It also indicates that maintaining an early vigor in growing field is crucial to increase the yield potential. In this research, various genotype performances of each trait of each accession was analyzed over years. G x E interaction was shown in various traits in CCPs. For flag leaf characteristics, the phenotypic performance of each CCP generally showed declining patterns over years. Also, the year effect in flag leaf characteristics (length and width) was much higher than other traits. It indicates that the phenotypic performance of flag leaf characteristics are more dependent on the environment and that the flag leaf characteristics are not stable traits under environmental change. On the contrary, it was shown that late and reproductive traits are more stable than early agronomic traits. In addition, higher heritability was shown in late and reproductive traits such as plant height and yield components. It can be inferred that in the early growing stage, environmental factors highly affect early agronomic traits. However, the heritability of the final yield (ton·ha^{-1}) was much lower than the yield components. Also, there was no G x E interaction between the genotype and year. Therefore, yield (ton·ha^{-1}) differences among CCPs were equal from one year to another. It indicates that the yield (ton·ha^{-1}) in CCPs might be more affected by environmental conditions. However, genotype differences over years might not fully explain the performance of CCPs as environmental conditions are continuously changing. In this research, the CCPs and *Naturastar (C)* showed a higher adaptability than *Julius (K)* in the Finlay-Wilkinson model. It indicates that the CCPs and *Naturastar (C*) have a higher adaptability across a wider range of different environments compared to Julius (K), which showed a lower adaptability and even a negative value in flag leaf length and spike fertility (%). It indicates that *Julius (K)* is not always able to adapt themselves to low-input farming systems. Also, stability was calculated by *root-mean-square error* (RMSE). A low stability value indicates little variation over environments and/or years because the closer the observed value is to the regression line, the smaller the deviation between observed and predicted value. Although the phenotypic performance showed declining or fluctuating patterns in various traits over years in CCPs and its populations, a higher adaptability and stability were shown over years. This research shows that CCPs have a high adaptability across various conditions with high stability. Therefore, in wheat production, evolutionary breeding can be suggested as a new paradigm, which brings more sustainability with higher genetic diversity under low-input farming systems. '*Relying on the intensive use of genetic variation, not intensive selection'* will be a new paradigm shift in the future wheat breeding. Finally, several limitations were also observed in CCPs. Therefore, it will be important to have a strategy that not only fortifies strengths and improves opportunities of CCPs, but also to reduce weaknesses and eliminate threats through consistent evolutionary breeding.

- ***Keywords:*** *wheat, CCPs, biodiversity, evolutionary breeding, heritability, adaptability, stability, low-input farming system, Finlay-Wilkinson regression model, GxE interaction*

Index

1. Introduction

Wheat, rice and maize are the major staple crops all around the world for humans and also principle resources which gave rise to the initiation of human civilization (Carver, 2009). These days, the production quantity of those three cereal crops in the world is approximately 3 billion tonnes in 2014. This amount accounts for more than 90% of all whole cereal crops (FAOSTATS 2016). Among the three major cereal crops, wheat (*Triticum aestivum* L.) shows the lowest production quantity (ca. 0.9 billion tones) but requires the largest production area, making its production more energy intensive (Hoad *et al.,* 2012). In the past, wheat was a crucial asset for humans in the settlement of new land. Nowadays, it is a globalized commodity which have been traded on an international scale; in 2013, for example, 325 million tonnes at a value of 103 billion USD was traded internationally (FAOSTATS 2016). Wheat has also been involved in people's everyday lives, including urban lives. People consume wheat for breakfast without thinking too much about it. In this respect, wheat is indispensable for human diet and nutrition. Remarkably, however, compared with maize and rice, wheat needs the largest acreage but produces the lowest quantity. It indicates that wheat is highly required to be under high-input cropping systems with favorable conditions to obtain high and stable yields (Costanzo *et al.,* 2016). However, climate change, environmental fluctuation and yield stagnation, etc., threaten wheat production and in the long term, the world food security. Climate change could have severe effects on wheat yields. Under fluctuating environments, its agronomic performance cannot be stabilized (Döring *et al.,* 2015). Also, wheat production would be constrained by diminishing returns to high-input cropping systems and eventually causes yield stagnation at one point (FAO 2016).

Nowadays, a new paradigm shift is highly encouraged to compensate these constraints in wheat production. So far, with the help of molecular breeding technology, diverse accessions containing genes of interests could be detected much more efficiently than before. As a consequence, the current approach of plant breeding has contributed to high yields with its corresponding grain quality in wheat production. As a result of global warming and/or climate change, however, environmental variability could have more pronounce influences on wheat production in the future. Conventional plant breeding is mostly conducted in well-managed and controlled growing conditions under high-inputs. Actually, those well-designed homogenous accessions could not be highly buffered against environmental variability or unpredictable (a)biotic stress factors when environmental changes are confronted (Döring *et al.,* 2015, 2011).

In contrast of conventional breeding, low-input organic breeding includes extensive breeding methods, minimizing external inputs. Organic agriculture is required to have an integrated management and rules out the use of synthetic chemical materials. This low-input cropping system could lead wheat to be exposed to more diverse and somewhat severe growing conditions. As a consequence, it is highly expected that the adaptability of wheat across environmental variability will be improved. However, it only allows a few accessions to be selected in those growing conditions as most accessions were developed at conventional farming systems with high-inputs. It means that its agronomic performances are usually optimized in high-input conditions. Therefore, the organic agriculture is highly required to obtain robust accessions which are able to be adapted to low-input conditions with yield stability. Also, under organic breeding, accessions are required to stand against (a)biotic stress caused by low-input conditions (Lammerts van Bueren *et al.,* 2008; Kokare *et al.,* 2014). It indicates that organic agriculture could have the problem of a lack of promising accessions adapted to the environmental variability. In addition, low nitrogen (N) supply in wheat production could cause deficiency of nutrients in wheat grains. This nutrient stress would directly affect baking quality of wheat and eventually, diminish its commercial value. However, provided that various accessions with diverse genetic variation are mixed into a population and naturally selected over time, the population that has high diversity could be buffered effectively against environmental changes (Muellner *et al., 2014;* Döring *et al.,* 2011). Therefore, the current breeding strategy is shifting from a high-input cropping systems to a low-input organic breeding. Particularly, in Europe, the cropping system is progressively moving towards organic and sustainable, low-input farming systems (Lammerts van Bueren *et al.,* 2008). Eventually, in wheat production, genetic diversity which is able to adapt to the environmental variability could be a new paradigm in the future.

1.1. Wheat Production

Nearly, all modern wheat accessions belong to two polyploid species: hexaploid bread wheat [*T.aestivum* (2n=6x=42, AABBCC)] and tetraploid hard-durum type wheat [*T.turgidum* L. (2n=4x=28, AABB)]. Bread and durum wheat are extensively grown all around the world. However, most of the total cultivated area of wheat production is usually sown to bread wheat. On the other hand, durum wheat is a minor crop, grown on ca. 8 to 10% of all wheat growing area (CIMMYT, 2001). Also, wheat is commonly divided into two types; winter and spring wheat. These two wheat types have different growing seasons according to its own growth habit. For example, when based on the geography and climate conditions of the Netherlands, winter wheat is sown around

September to October and harvested around late July the next year with a life cycle of ca. 9~10 months. On the other hand, after March, It is possible to sow the spring wheat and according to the weather conditions, harvested after ca. 4~5 months. (http://www.natuurkalender.nl). It is known that wheat grown in the Netherlands are mostly winter wheat and only a small part of it is spring wheat as winter wheat maximizes grain yield for the feed industry rather than for bread baking quality (CBS, 2012; Osman, 2014). On the contrary, spring wheat is more preferable under organic farming systems because of a better baking quality. Winter wheat production with different chemical treatments and farming practices in each season is called *Wheat Production Decision Matrix* (Table 1).

Wheat is an autogamous crop based on the genetic structure of self-pollinated populations. it is known that natural cross-pollination occurs for about 1~4% in wheat production (Acquaah 2009). However, heterozygosity is reduced by 50% with each consecutive generation of self-pollination [heterozygosity: $(1/2)^n$; n=number of selfed generation], which means that the proportion of heterozygosity remaining in a population is very small over time (Sleper and Poehlman, 2006). Therefore, it is possible for wheat to have open pollinated varieties OPVs and seeds can be sown again by farmers for following season like landraces due to Farmers' Rights. Furthermore, the plant breeder’s rights (PBRs) gives breeders exclusive control over the propagated and harvested material of a new variety. PBRs can be generally applied in wheat as the criteria is satisfied; distinct, uniform and stable (UPOV, 1991)

1.2. Wheat Breeding program

The objective of wheat breeding is creating new genotypes that contributes to a high yield potential, stability and improved quality. Therefore, it is important for breeders to decide genetic improvements to reach breeding goals. A high yield potential can be obtained by crossing high-yield genotypes and selecting transgressive segregates. The ability of the plant genotype to exhibit consistent yield potential across a wide range of environments can be defined as yield stability, which implies minimum yield reduction from environmental changes. Finally, grain quality is directly related to baking quality which indicates market value (Sleper and Poehlman, 2006). In this respect, several specific breeding selection methods for autogamous crops are currently available.

TABLE 1. Wheat Production Decision Matrix[1] of winter wheat (*T.aestivum*) in Netherlands

Growth stage	Pre-sowing	Sowing	Early stage	Mid stage	Late stage	Harvest
		Seedling	Tillering	Stem extension	Heading	Ripening
Season		Oct~	~Apr	May~	June~	late July~Aug
Overall strategy	▪ Choice of seed varieties; ▪ Seed germination test; ▪ Field preparation	▪ Monitoring seedling emergence/ development ▪ Control of soil-borne disease	▪ Monitoring wet injury; ▪ Early weed control; ▪ Monitoring tillering	▪ Insect/disease control; ▪ Weed control; ▪ Monitoring nutrient deficiency / physiological stress; ▪ Keeping possible irrigation systems in no-rain conditions; ▪ Preventing lodging		▪ Monitoring grain maturing; ▪ Monitoring crop lodging; ▪ Seed cleaning ▪ Recording yield and quality
Chemical decision	▪ Basal fertilizer (NPK) application	▪ Nematicide application		▪ Germicide (powdery mildew, leaf rust etc.) application; ▪ Additional fertilizer (NK) application		▪ Grain fumigation
Farming practices	▪ Disinfection of seeds; ▪ EC/pH check (soil); ▪ Tillage; ▪ Preparing furrow beds;	▪ Off-type elimination (roguing); ▪ Drain maintenance; ▪ Monitoring frost damage; ▪ Monitoring 1st tillering; ▪ Weed management; ▪ harrowing the field		▪ Monitoring crop lodging ▪ Control of effective tiller number	▪ Monitoring 1st flowering; ▪ Monitoring pollination/ fertilization	▪ Machinery Inspection; ▪ Preventing grain shattering ▪ Seeds saving for next season

[1] Based on conventional wheat production. *(Adopted/modified from Hoad et al., 2012)*

1.2.1. Mass selection

Mass selection is known as the most simple selection method in breeding programs. It is also known for being the most suitable method for autogamous crops. For mass selection, the first step is to compose a base population which is a mixture of genotypes [(A*B)+(C*D)+(E*F)…]. Followed by selecting and harvesting (in bulk) to sow as a mixture in the next season. This way, several cycles would be repeated in consecutive seasons to increase the frequency of desirable traits. Mass selection is divided into a positive and negative selection. Positive selection is to select wheat plants containing desirable traits individually, based on phenotype in a population and harvest in bulk to produce the next generation's population (Figure 1 A). On the contrary, the method to discard undesirable traits in wheat and harvest the remaining plants in bulk is called negative selection. Besides those artificial selections, natural selection is also involved in mass selection. Active artificial selections are conducted by breeders but growing the populations in certain environments also leads to natural selection pressures to increase the frequency of genotypes with desirable traits. So, natural selection will complement the artificial selection in mass selection. This method is simple and easy to control in bulk. In addition, the final homogeneous population could be well-adapted to the local conditions. However, if accessions have a low heritability, mass selection would not be a suitable method to select desirable traits as the phenotype is not highly represented by its genotype and is more affected by environmental conditions. Therefore, developing accessions which can buffer non-genetic variation relative to genetic variation to increase stability could be a crucial factor in wheat breeding.

1.2.2. Pure line selection

Pure line selection is a similar method as the positive mass selection, the difference is that the selected wheat plants are harvested separately and the seeds per plant are also kept separately. In the next season, the seeds per plant are sown and evaluated by breeders on the basis of the planting row. In this case, each row would be the criteria to be evaluated, not each single plant as all plants in a row are derived from a homozygous parent. It means that those are genetically equal and its phenotypic difference is only due to non-genetic variations (pure lines = heritability is 0). Rows showing low performance and high variability are discarded and the remaining rows are harvested in bulk (Figure 1 B). Finally, each harvested row in bulk is tested several years and the best pure line will be released as accessions or as new parents in the breeding program.

1.2.3. Pedigree selection

Pedigree selection is the most common selection method in autogamous crops. Two promising homozygous parents (P_1/P_2) are selected by breeders and new traits from P_2 are introduced to P_1 as emasculating pollens in P_1, to generate F_1 population which is a new combination but not segregated yet. Since F_2 population, wheat plants containing desirable traits are selected and harvested individually as F_2 population is supposed to be segregated. Those individual wheat plants are sown in each planting row to make the F_3 population and within the row, wheat plants containing desirable traits are selected and harvested individually. On the contrary, planting rows which show undesirable traits or segregation are completely discarded. This way, the same procedure is repeated and the F_7 population will be obtained (Figure 1 C). As generations go by, wheat plants become more homogeneous with the increase of homozygosity. However, if wheat plants containing desirable traits have a low stability, individual wheat plants are not eligible to be evaluated exactly on the basis of genotypic variation. The main difference between the pure line and pedigree selection can be explained as individual selection and bulk selection. For the pure line selection, each favorable trait is selected and sown again but it will be harvested in bulk in the next season. On the contrary, pedigree selection is based on each single favorable trait. Like in the pure line selection, at first, each individual plant is selected and sown again. In the next generation, each single favorable plant is harvested and sown individually within several cycles in order to increase homozygosity among plants with favorable traits.

1.2.4. Recurrent selection

Recurrent selection aims to increase the frequency of desirable traits of several parents and upgrade its germplasm. First of all, several homozygous parents are randomly intercrossed to generate a F_1 population which will be intercrossed again. In this case, three-way $[(P_1xP_2)xP_3]$ or double-cross $[(P_1xP_2)x(P_3xP_4)]$ breeding would be exerted. Those new segregating combinations are sown as a mixture and grown in F_2 population. Each plant in the population containing favorable traits will be selected and harvested individually and sown in a row for another generation. Also, planting rows which show undesirable traits are completely discarded. The selected rows are harvested in bulk and sown again as a bulk population. This new population reverts to the recurrent selection for its second cycle (Figure 1 D) and the procedure will be repeated in several cycles to increase the frequency of favorable traits. However, effective intercrossing is only based on simultaneously flowering time among wheat plants. Therefore, flowering time which inhibits genetic variation might result in assortative mating.

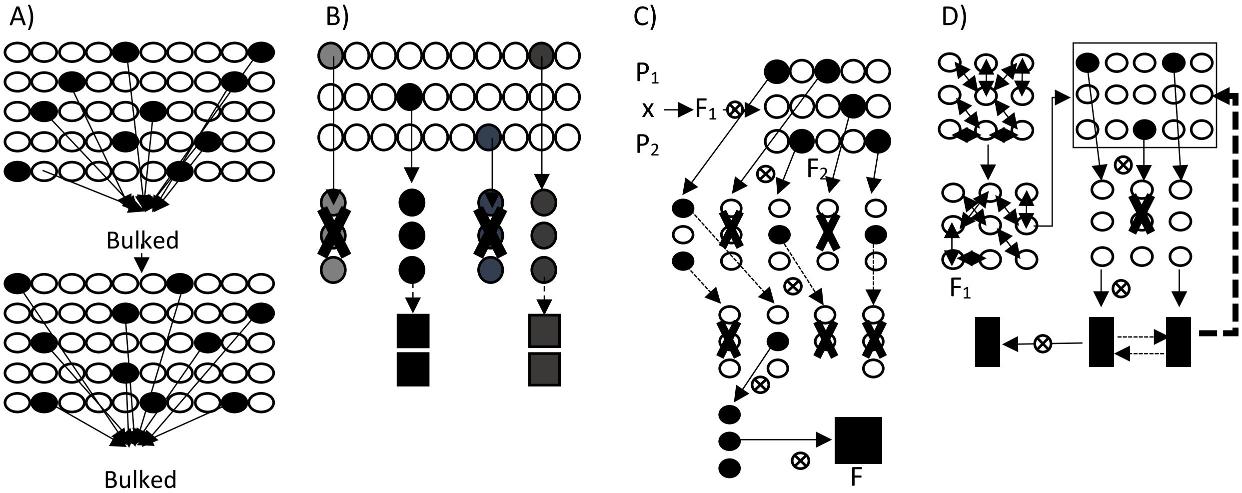


FIGURE 1. **General wheat breeding program.** A) Positive mass selection; Selected wheat plants (black dots) are harvested and mixed to be sown again B) Pure line selection; Selected wheat plants (black dots) are harvested and sown in a separated row. Each selected row is harvested in bulk (row) C) Pedigree selection; In each generation, wheat plants containing desirable traits (black dots) are continuously selected but each row containing undesirable traits is discarded D) Recurrent selection; After final intercrossing, the new population starts the second cycle of the recurrent selection to upgrade new germplasm

1.3. Evolutionary breeding in wheat

Conventional wheat breeding programs are usually based on a primary strategy to select genotypes containing desirable traits which are well-adapted to both abiotic and biotic conditions. Through the selection, much more homogeneous accessions with a high level of homozygosity are able to be produced in well-managed environmental conditions. Given that the phenotype does not highly represent its genotype under low heritability conditions, optimum environmental conditions are intensively required in order to reduce environmental errors (non-genetic variation) under the high-input farming systems. However, in this conventional approach, climate change or reduction of external inputs would result in uncertainty in the crop performance because of a higher Genotype x Environment (G x E) interaction (Costanzo *et al.,* 2014). G x E interaction means that the genotype differences in phenotypic response(s) are dependent on the environment. It can be shown as the statistical model for the phenotypic variance [V_p]. The statistical model describing the phenotypic value [P] of genotype [G] in environment [E], allowing for interaction [G x E] and experimental error [e] would be: $V_P = V_G + V_E + V_{GE} + V_e$. However, in the Finlay-Wilkinson regression, this statistical model can be also shown as $V_P = V_G + \beta_E + e$ in case the environmental variables are not fully recognized in many Multi-environment trials (METs)

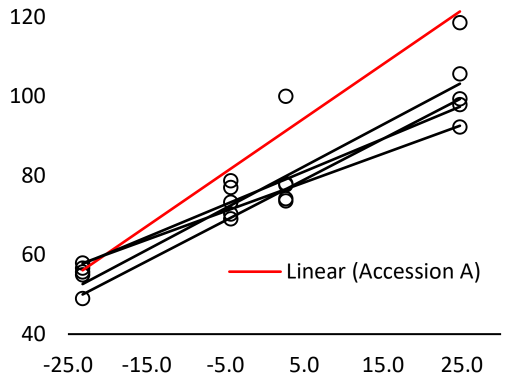


FIGURE 2. Finlay-Wilkinson Regression model. In this simulation, wheat accession A shows the steepest slope. It indicates accession A has a broad adaptation across a wide range of environments compared with other accessions. (x-axis: environmental index, y-axis: yield)

(reviewed by Maliepaard, 2015). This approach is to characterize each environment as an environmental index by the average performance of all genotypes. Provided that the performance of the individual genotypes over the environmental index is drawn as a plot, a linear regression model can be observed (Figure 2). In this regression model, the slope [β] of each genotype presents its adaptability in different environments. If the slope is steep, it indicates a high responsiveness is shown which indicates a genotype performance across different environments is good. In plant breeding, a high adaptability is required as it can be grown across a wide range of environments and is expected to have a superior performance with little variation (reviewed by Maliepaard, 2014). By extension, variations due to deviation from regression (s^2d) can also be used as parameters of adaptability and stability. In this case, a cultivar with a low (s^2d) value is considered to be highly stable. Therefore, adaptability across a wide range of environments can be defined by three values: (1) average grain yield, (2) the slope in the Finlay-Wilkinson regression and (3) the deviation from the regression line (s^2d) (Kokare *et al.,* 2014). In terms of the model; $V_P = V_G + \beta_E + e$, the prerequisite of an acceptable accessions for adaptability across a wide range of environments is to show an overall genotype performance and a high buffering capacity against environmental changes in order to reduce environmental errors (non-genetic variation). However, current conventional breeding approaches in wheat have constraints to fulfill the prerequisite as it aims to increase homozygous lines. Although mass or pure line selection is the method to use extant genetic variation and also pedigree or recurrent selection is the method to create a new variation using crossing, the final goal of those methods is to narrow down the variation and fix a new homozygous cultivar with high homogeneity. In autogamous crops, the current strategy to exploit the variation is by selecting genotypes which are well-adapted to certain controlled environmental condition or by narrowing down genetic variation to increase genetically identical individuals (Döring *et al.,* 2011). However, diversification at genetic levels could improve the ability of crops to stand against a wide range of environments and various growing condition(s) within one season and location. In evolutionary breeding, evolving crop populations have the capability to adapt to various growing conditions as a high level of genetic diversity in crop populations is mostly created under the force of natural selection.

The development of resilience of evolving crop populations against environmental variation should be a major strategy in wheat breeding under the low-input (Costanzo *et al.,* 2016; Döring *et al.,* 2011).

1.3.1. Agrobiodiversity

Due to the negative impacts of intensive selection, the decline of genetic diversity has increased the dependence of crop yield on high-input farming system. Current breeding is mostly performed under high-inputs and has the low opportunity to exploit genetic diversity at low inputs. (Phillips and Wolfe, 2005). These days, the loss of agrobiodiversity driven by high-inputs is evaluated to be the similar effect expected from climate change as the loss of agrobiodiversity is more likely to be affected by inputs than to yield per se (Emmerson *et al.,* 2016). However, these days, as the paradigm of being more sustainable with reduced external inputs stands out, a new strategy in plant breeding program is suggested; relying on the intensive use of genetic variation, not intensive selection (Østergård *et al.,* 2009; Costanzo *et al.,* 2014). Therefore, genetic diversity within a population is an essential source of biodiversity and it can be defined as quantifying the extent of genetic variability (Table 2) (Hughes *et al.,* 2008). Genetically diverse populations could change its genetic makeup over time by natural selection and gradually increase resilience in environmental changes. Considering factors acting on genetic diversity, the most efficient way to increase diversification at the genetic levels in populations could be composite cross populations (CPPs) as an example of evolutionary breeding.

TABLE 2. The five main factors acting on genetic diversity in populations

Factor for genetic diversity	Quantification of genetic variability
Genotype abundance	The number of genotypes within a population.
Heterozygosity	The proportion of loci that carries two different alleles at a one locus within one cultivar.
Genetic variance (V_G)	The variance in a phenotypic trait due to genetic differences.
Adaptibility[1]	The flexibility of a genotype in its response to environments.
Heritability (h^2)	The ratio of the genetic variance to the total phenotypic variance (genetic variance + non-genetic variance) in the population.

[1] Adaptability can be calculated as a regression coefficient of environmental index or variation due to deviation from regression (s^2d)

1.3.2. Composite Cross Populations (CPPs)

Unlike conventional breeding programs which select promising individual genotypes in each generation, for composite cross populations (CPPs), the whole population is consistently exposed to natural selection in each subsequent generation. CCPs recognizes the importance of a Darwinian view and evolutionary processes with sustainable agricultural practices (Jones *et al.,* 2005; Phillips and Wolfe, 2005). The general procedure of CPPs is commonly composed of four stages; (1) A base population with genetic diversity is created by crossing multiple promising parents, (2) the harvested seed from the crossings is mixed evenly in bulk to produce the 1st CCP generation, (3) the harvested seed from 1st CCP generation is saved without artificial selection of promising individuals and (4) the genetically diverse seeds of CCPs are not only used as food or feed but also exploited as a new genetic material in further breeding programs for new composite crosses (Figure 3). The effect of CPPs can be defined in terms of 4Cs; Complementation, Cooperation, Compensation and Capacity. It is known that the use of varietal mixtures in a population could complement each other for certain beneficial traits such as nutrient availability, water use efficiency and high photosynthesis activity. For cooperation, it is known that more effective triggering of induced resistance was present in diversified populations. This mechanism is called allelopathy (Finckh 2009). In terms of allelic diversity, it is known that heterogeneous crop populations such as CCPs show more capacity to cope with climate variability than homogeneous pure lines because of genetic variance (V_G) increase due to broader frequency distribution of genotype performance across environmental changes (Østergård *et al.,* 2009; Döring *et al.,* 2011; Costanzo *et al.,* 2014). Also, these advantages of CCPs can be explained by the response to selection (R). For example, if the phenotypic variance is limited due to narrow genetic diversity, the genetic variance (V_G) selected from the population will also be limited. That is, a large phenotypic variance could provide a wide range of genetic variance (V_G) from which to select (Acquaah 2009). Under the narrow phenotypic variance, selection for high performance is limited. It means that the selection differential (S) and the response to selection (R) are also reduced compared to the large phenotypic variance (Figure 4). In terms of breeder's equation; $R = h^2$ x S, the response to selection (R) can be defined as

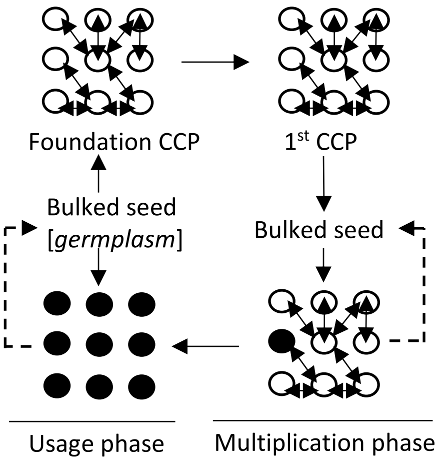


FIGURE 3. The stage of evolutionary plant breeding. Evolutionary breeding also includes conventional breeding methods at the first stage but is more focused on varietal mixtures without active selection of individual plants since second stage.

genetic advance (ΔG). It indicates that as one generation has passed, ΔG can increase when the selection differential (S) becomes larger with high heritability (h^2). If h^2 is 1, the mean of offspring would be equal to the mean of the selected parents (R=S). On the contrary, if h^2 is 0, there would be no progress at all (R=0). Therefore, in order to increase ΔG, it is necessary to obtain wide genetic diversity with accessions containing high h^2. In this respect, genetic diversity generated by CCPs within a population is able to increase ΔG and its evolutionary fitness.

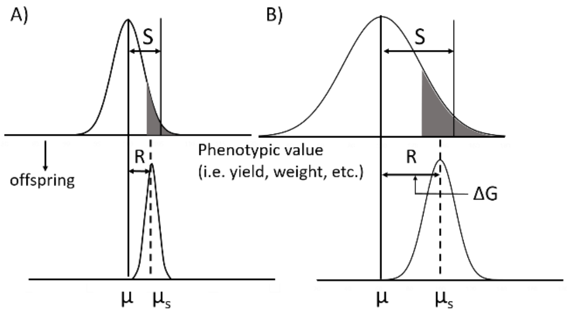


FIGURE 4. The effect of phenotypic variance on genetic advance. (A) Small phenotypic variance. (B) Large phenotypic variance. When the phenotypic variance is large, genetic advance (ΔG) increases and vice versa.

1.4. Breeding for organic and low-input cropping system

In the organic farming system, it is assumed that a high level of genetic diversity would be required in a population to adapt themselves to varying low-input cropping systems. Many researches have already indicated that genetic diversity in the field is particularly strengthened in organic and low-input farming systems because of the expanded heterogeneity of environments over time. As a consequence, the adaptability across a wide range of environments could increase in low-input systems as genetically heterogeneous populations are able to buffer the impacts of environmental changes (Dawson *et al.,* 2012). Eventually, it indicates that agrobiodiversity in a population is an essential factor for crops to cope with environmental changes. Nowadays, however, modern and intensive agriculture has reduced agrobiodiversity. In the past, crops were naturally grown as heterogeneous landraces which adapt themselves to various environments, maintaining genetic diversity. On the contrary, controlling growing conditions through high-external inputs, monocultures and current breeding methods such as the pure line or F_1 hybrid have caused more uniform crops and narrow genetic diversity as consequences. Wheat is an autogamous crop and its breeding approaches are to increase homozygosity for certain traits. Furthermore, a high-input system under homogenized growing environments in wheat production makes it reliant upon specific elite varieties (Frison *et al.,* 2011; Dawson *et al.,* 2012). This reliance on narrow genetic base is more vulnerable to unpredictable environmental changes. What is more, in organic farming systems, certain traits such as resistance to mechanical harrowing, ground cover rate or weed suppression ability are much more

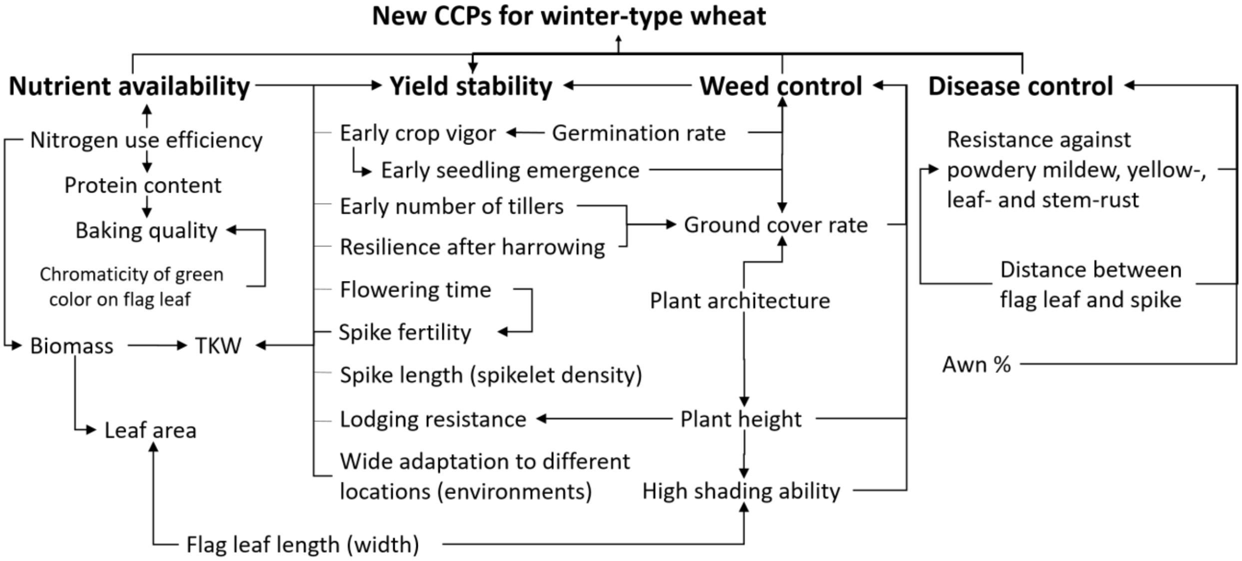


FIGURE 5. Interaction of favorable traits for winter-type wheat under organic wheat breeding. It is hypothesized that promising new CCPs are interactively affected by several traits, not a single trait. Arrowhead indicates former trait(s) affects latter trait(s).

considered as important factors compared to in conventional systems (Löschenberger *et al.,* 2008). Therefore, it is highly required to develop new breeding approaches which are able to maintain a high level of genetic diversity with favorable traits (Figure 5).

1.4.1. Nutrient availability

Under organic farming systems, substitutes for chemical treatments are highly required to maintain crop yield and prevent nutrient deficiency. In organic agriculture, chemical fertilizers are usually replaced by manure or other organic fertilizers. In case of manure, the general composition of each animal manure was already investigated and it is generally known that ca. 78.5kg·ha^{-1} N is generally required to produce winter wheat (Edwards *et al.,* 2006). Therefore, it is possible to calculate the total amount of manure according to each manure type (Table 3). For example, in order to supply 78.5 kg·ha^{-1} N, for poultry manure, the amount of 5.2 ton·ha^{-1} is generally required to be applied to the field of winter wheat. While applying manure for adequate N supply, however, phosphorus (P) is also highly supplied, for example, 114kg·ha^{-1} for poultry manure. Phosphorus shows the lowest movement among nutrients in soil and a continuous application of phosphorus surplus via animal manure would cause an accumulation of insoluble phosphoric acid which results in salinization in soil. Alternatively, organic fertilizers such as oil seed cake or castor oil are also able to be used under organic farming system. In the case of a commercial organic fertilizer (i.e. 20kg·bag^{-1}, 4-2-1), 100 bags·ha^{-1} would be enough to supply

TABLE 3. The nutrient composition of animal manure and requirement of total N amount

Manure Type[1]	Composition of manure ($kg \cdot ton^{-1}$) [a]			Total amount ($kg \cdot ha^{-1}$) [b]			Total [c] (ton)
	N	P	K	N	P	K	
Poultry	15.0	21.8	15.4	78.5	114.0	80.6	5.2
Beef	9.5	6.4	10.4		52.9	85.9	8.3
Sheep	8.2	5.0	11.8		47.8	112.9	9.6
Swine	4.5	4.1	3.6		71.5	62.8	17.4
Dairy	4.1	1.8	4.5		34.4	86.1	19.1

[1] All values are on a fresh weight basis and no straw, [a] Adopted/modified from Rosen and Bierman, 2005. [b] NPK amount included in [c] total manure amount for supplying adequate amount of $N \cdot ha^{-1}$ in winter wheat.

an adequate amount of N. In addition, humic acid is known for major organic constituents of humus. The decomposition of plant and animal remains in soil, leads to biological process and the accumulated carbon in soil is converted into stable humus which contributes to high organic matter contents (reviewed by Stevenson, 1994). Therefore, humic acid could also be a good substitute for chemical fertilizers. The more available nutrients in organic agriculture, the more the various substitutes for chemical treatments could be applied. Therefore, in order to increase the nutrient availability, it will be an important goal to select promising accessions which shows a higher ability to uptake nutrients under low-input organic breeding. In this case, nitrogen-use efficiency (NUE) could be considered as an important trait in accessions. NUE, defined by Moll *et al.* (1982) can be explained as the amount of grain dry matter produced per unit of available nitrogen (N) from the soil and fertilizer.

Most accessions developed under high-input systems and conventionally managed systems may not possess traits in which NUE is optimized in organically managed systems. Eventually, wheat accessions which show top performance in conventional systems will not exhibit the same performance in organic systems. Therefore, in order to identify which accessions are able to show high performance and which traits are important for high yield potential at low N input, new breeding strategies are required in organically managed systems (Gaju *et al.,* 2011; Dawson *et al.,* 2011). Since it is difficult to measure NUE quantitatively, one strategy is to measure traits contributing to NUE. Under the low-input farming system, the chromaticity of green color on flag leaf could be a crucial trait in evaluating NUE and protein content in grains which directly affects baking quality. Nitrogen deficiency mainly shows leaf yellowing, starting with older leaves. Nutrient availability and translocation will increase in a plant when accessions show high NUE. Therefore, NUE could be expected through the degree of green color on flag leaf and duration of green color. Leaf area is also one of the important criteria to evaluate nutrient availability. In this case, formula suggested by Stickler *et al.,* (1961) can be used due to the difficulty to measure the actual leaf area at senescence stage (BBCH-scale: 92 to 99).

1.4.2. Yield stability

Yield stability is a comprehensive concept to explain yield potential. Generally, yield stability indicates a genotype performance that does not change across environments. Genotypes are considered as stable if it only shows slight deviations in the genotype performance across various growing conditions (Mühleisen *et al.,*2014). While yield loss is one of the constraints in organic agriculture, higher buffering capacities across a broad range of environments are shown in organic agriculture compared to conventional agriculture as those are generally developed under large variabilities with low input and have a higher adaptability against changing environmental conditions (Wolfe *et al.,*2008). In organic agriculture, for winter wheat, there are several favorable traits to affect yield stability and it is usually affected by several traits, not a single trait. For example, traits such as early plant density, the number of tillers or resilience after harrowing can be considered as important characteristics of winter wheat at the tillering stage (BBCH-scale: 20 to 29). In addition, in terms of METs, adaptability to different locations (or environments) can be assessed by comparing the yield potential data of previous years in different locations.

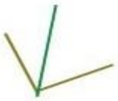
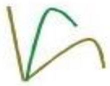
1.4.3. Weed competitiveness

Effective weed management is a crucial farming practice for outstanding organic agriculture because under the organic farming system, low weed control is the main constraint. In addition, the ability of wheat to compete against weeds is difficult to be obtained under organic conditions as less strategies and/or opportunities are available for weed control (Mason *et al.,* 2006). Crop competitive ability against weeds is usually considered in two ways: (a) the capability of crops to tolerate weed pressure by maintaining grain yield and (b) the capability of crops to suppress weed growth (Coleman *et al.,* 2001). The competence to suppress weeds under organic conditions is strongly affected by the interaction among favorable traits, not a single trait (Figure 5). Excepting favorable traits in accessions, other elements could be also considered as important elements for weed control. According to previous researches, allelopathy and harrowing also play important roles in weed control (Donner *et al.,* 2006). Those elements are interacted with each other and the interaction plays a pivotal role in weed competitiveness in organic farming system.

In organically managed systems, the first priority for effective weed control is to secure promising accessions containing favorable traits to suppress weeds. In the winter wheat, there are several traits related to weed control. First of all, the ground cover rate can be a crucial characteristic in wheat. The ground cover rate strongly affects weed growth and also promotes light interception of the crop. Also, a good ground cover increases the shading ability which can improve weed control without high inputs and negative environmental impacts. Therefore, the ground cover rate is an effective indicator of the shading ability and furthermore, of high yield components (Donner *et al.,* 2006; Drews *et al.,* 2009). Also, high tillering ability will form high plant density in a population. As a single trait, high tillering might not be always a good indicator of competitive ability. However, the tillering capacity could be the most important trait at low density of plant populations because tillering capacity is defined as an availability to compensate for low vigorous growth with extra tillers. Therefore, selected accessions in organic breeding should have high tillering capacity to cope with varying growing conditions in order to secure a stable yield potential and promote weed suppression (Hoad *et al.,* 2006; Osman *et al.,* 2015). Rapid early growth to stem extension also helps the crop to maintain a light interception and shading ability of the crop to suppress weeds.

There are several traits conferring early growth and they are generally known as seed size, germination or seedling growth rate, tillering development, etc. (reviewed by Hoad *et al.,* 2012). Furthermore, rapid early growth of shoot is highly correlated with root development as early root development shows a pivotal role in the use of water and nutrients during the early stage of crop growth (Donner *et al.,* 2006). On the contrary, the plant height itself is not a solid evidence to increase the crop's competitive ability against weeds because a tall straw itself is not as important as early stem elongation except for by-product such as compost. However, the plant height is

FIGURE 6. A five-point scale for describing plant growth habit for organic farming

Curvature	Erectophile		Intermediate	Planophile	
	Narrow	Spread		Spread	Flat
Plant habit	1	2	3	4	5
Straight					
Curved					
Leaf angle	< 15°	< 30°	wide range	30 ~ 60°	>60°

Adopted /modified from Hoad et al., 2006 generally considered as a favorable trait for enhancing weed competitiveness. Particularly, previous research indicates that tall accessions are the most competitive when grown in high plant population densities or in a condition of non erect-leaf habit. It suggests that the plant height is highly interacted with other factors which contribute to weed suppression (Donner *et al.,* 2006; Hoad *et al.,* 2012). Finally, the plant growth habit is the trait associated with a high ground cover rate (Figure 6). It is known that an early non erect-leaf habit at the tillering stage (BBCH-scale: 20 to 29) can be a good indicator of high weed competitiveness. The plant growth habit is defined as leaf inclination or angle. Previous research indicates that non erect-leaf accessions show a higher weed suppression than erect-leaf accessions because of a high ground cover rate and increased light interception. The ground cover rate at the tillering stage is highly correlated with weed suppression throughout the entire growing season (Wolfe *et al.,* 2008; Hoad *et al.,* 2012; Hoad *et al.,* 2006). Therefore, a non erect-leaf habit can be considered as an important trait in genotype selection under organic breeding.

In organic agriculture, harrowing is regularly conducted as a mechanical weed control. Harrowing is a common method for weed control and repeated for several times for sufficient weed control, particularly in row crops (Van der Weide *et al.,* 2008). Harrowing also breaks the crust of the surface soil and allows oxygen to enter the soil, which allows aerobic microbes to be highly activated in soil. However, in case of sensitive crops with a weak root system, harrowing will damage the root system. Therefore, for effective weed control in organic agriculture, accessions containing a high competitive ability against weeds are highly desirable and it could be one of the main objectives of organic wheat breeding.

1.4.4 Disease assessment

For the winter wheat, several diseases such as powdery mildew, yellow rust and septoria Tritici Blotch (STB) are known as the most serious diseases which cause direct yield loss (Figure 7, Table 4). Under organic farming systems, a low productivity of winter wheat due to pest damage could be the biggest problem as it is not allowed to use any pesticides or insecticides. The best way to prevent or minimize low productivity in organic farming is to choose well-developed accessions against diseases. In the long term, however, enhancing agroecosystem services which are ecological functions provided by nature, such as biological pest control will be a crucial approach because in the future, agriculture will require more sustainable farming systems by replacing external inputs with low-inputs within the agroecosystem. External high-inputs indicate the increased use of fertilizers, pesticides for cereal production which has been intensified by high external inputs (Schellhorn *et al.,* 2015; Emmerson *et al.,* 2016). In the future, however, more sustainable farming systems to control wheat diseases will be suggested under the low-input systems. Along with promising accessions and future agroecosystems, it is known that populations with functional diversity are able to be more resilient against diseases than genetically uniform populations. In CCPs, however, when the agronomic traits that increases fitness across a wide range of environments, are highly linked to susceptible genotypes, susceptible genotypes could be maintained in a population. On the other hand, if the fitness of susceptible genotypes is lower than resistant genotypes, the proportion of susceptible genotypes will decrease over time and its genetic diversity with favorable traits in a population can reduce the yield loss caused by pest damage pest damage in terms of 4Cs. (Döring *et al.,* 2011).

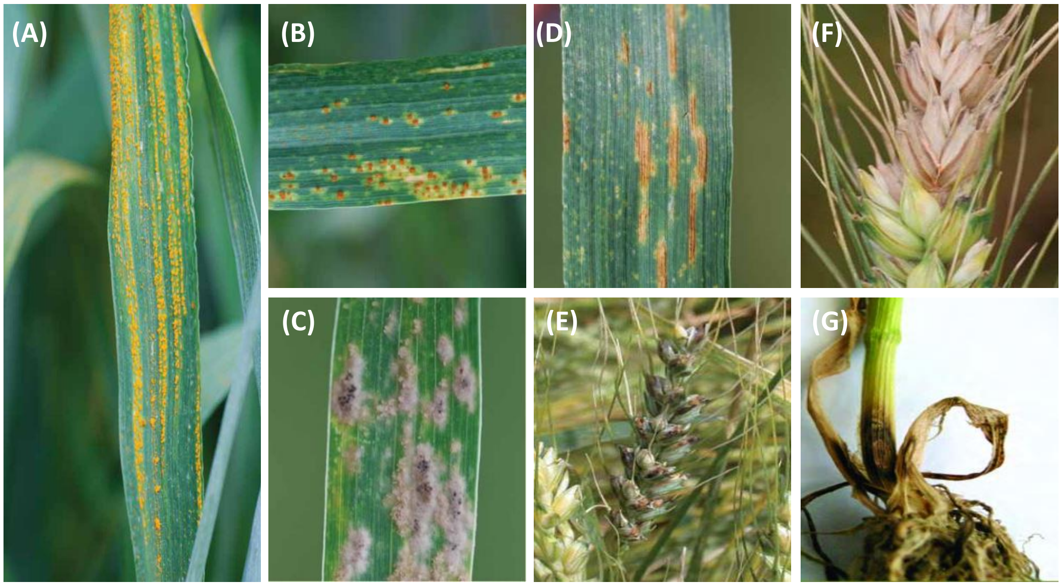


FIGURE 7. Common diseases in winter wheat. A) Yellow Rust B) Brown Rust C) Powdery Mildew D) Septoria Tritici Blotch E) Common Bunt F) Fusarium head blight G) Eyespot. † Symptom of each disease was described in **TABLE 4**.

TABLE 4. Common disease in winter wheat and symptoms

Infection	Name (Scientific name)	Routes of transmission	Life cycle	Symptoms
Leaves	Stripe Rust (*P. striiformis*); Yellow Rust	Caused by air-borne spores which may travel long distances	The disease develops rapidly when cool (10–15°C), damp weather with overnight dew or rain.	▪ Yellow to orange-yellow stripes (spores) on the leaves
	Leaf Rust (*P. triticina*); Brown Rust	Caused by air-borne spores which were survived from overwinter	The spore germination develops rapidly when surface moisture is on leaves followed by overnight dews with high temperatures (15–22°C)	▪ Scattered orange to orange-brown lesions (blister-like) on the leaves
	Powdery Mildew (*B. graminis*)	Developed from sexual and asexual spores produced on residue from previous crops	Air-borne spores produced from other crops spread widely and infect host plants particularly under the high humidity condition	▪ Pale gray, powdery colonies of mycelia on the surfaces of leaves, especially on lower leaves
	Septoria Tritici Blotch (*M. gramunicola*)	wheat residue from the previous year	Air-borne spores disperse by rain splash and physical contact between leaves	▪ Brown oval leaf spots (lesions) were observed. Water-soaked lesions gradually turn into brown
Head & grain	Common (*T. tritici*) and Dwarf bunt (*T. controversa*)	Seed-borne diseases	Spores germinate on the seed surface and infect seedlings. Visible symptoms appear after heading.	▪ Appearing after ears emerge; ▪ stunted and yellow streaks (flag leaf); ▪ grains replaced by bunt balls; ▪ giving off a fishy odor
	Fusarium head blight (*F. graminearum*)	Crop residues by rotation (stalk and ear rot of maize, stalk rot of sorghum, and scab of rice)	Fusarium attacks the spikes and the ovaries are infected at anthesis. It spreads from floret to floret by mycelial growth.	▪ occurring after flowering; ▪ prematurely bleached (spikelet); ▪ pink discoloration in kernels; ▪ shriveled scabby grains;
Stem & roots	Eyespot (*Rhizoctonia cerealis*)	Caused by conidia or mycelia produced on crop debris on the soil surface	Winter wheat is more frequently damaged in cool, moist climates.	▪ eye-shaped, elliptical lesions produced on the internodes of the lower stem

Adopted /modified from Duveiller et al., 2012 and Paveley et al., 2000

1.5 Problem statement

The idea of composite cross populations (CCPs) was initially introduced by Suneson (1956) as evolutionary breeding. This promising breeding method to increase resilience against environmental variations has been considered as a new paradigm in the face of fast climate change caused by global warming (Döring *et al.,* 2010). Conventional breeding methods are usually dependent on selecting genotypes under optimally managed environmental conditions. The conventional cropping system with a high level of external inputs have diminished agrobiodiversity. In the long term, it will bring yield stagnation which results from low genetic advance, loss of soil fertility and (a)biotic stress caused by environmental variations. Recently, FAO (2016) introduced two scenarios suggested by the International Food Policy Research Institute (IFPRI). The study indicates that, if there are no changes in current high input cropping systems, the price of cereals could rise substantially between 2010 and 2050. On the other hand, provided that new approaches to increase productivity sustainably are suggested, the resulting higher productivity would keep cereal prices in 2050 very close to those of 2010 (Figure 8). Therefore, evolutionary breeding can be a new approach to simultaneously stabilize crop productivity and pricing in the future.

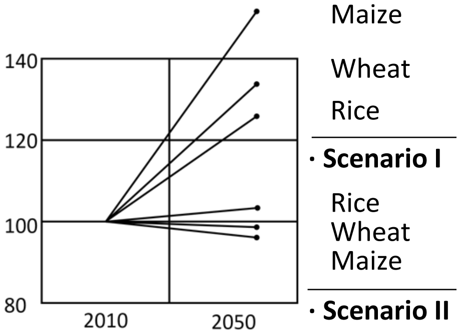


FIGURE 8. Expected changes in world cereal prices between 2010 and 2050 under two scenarios: Index (2010 = 100). Scenario I: No changes in current policies and investments. Scenario II: investment in increasing productivity sustainably (Adopted/modified from FAO 2016)

Composite cross populations (CCPs) are usually exposed to natural selection without any intervention by breeders in order to increase favorable traits naturally. Its physical mixture of different genotypes could provide an improved ability of crops to buffer environmental changes. Eventually, evolutionary breeding which over time increases crop diversity in a population could provide higher possibilities of enhancing crop performances in highly unpredictable environmental changes. Also, it will provide higher possibilities of stabilizing cereal prices with a high productivity in comparison with high input farming systems or monocultures (Döring *et al.,* 2010). However, even though composite cross populations (CCPs) show strengths in dealing with environmental changes and potential opportunities in the future, there are also several limitations (Table 5). First of all, due to low inputs, nutrient deficiency, low disease and weed control could be the main risk

TABLE 5. **SWOT analysis for composite cross populations**

Strengths	**Weaknesses**
▪ Wide adaptation across environmental conditions ▪ Co-evolution with nature due to consistent natural selection ▪ High resilience against environmental fluctuation due to genetic diversity ▪ Improving robust accessions or populations against disease (i.e., allelopathy) ▪ Development of weed-resistant cultivar ▪ Advantages of 4C; Complementation, Cooperation, Compensation and Capacity	▪ To be able to adapt to low-input farming systems (no herbicides, limited nitrogen): there is lack of nutrient efficient and weed suppressive parent lines to compose appropriate populations ▪ Low yield potential due to low inputs ▪ Low commercial value due to heterogeneity ▪ Difficulty to obtain plant variety rights due to non-uniformity
Opportunities	**Threats**
▪ Increase of genetic advance over time ▪ Sustainable agriculture through low input cropping system ▪ Creation of new germplasm for future breeding programs ▪ Stabilization of cereal price in the long term	▪ Poor baking quality due to lack of proteins ▪ Spreading epidemic disease due to no chemical use ▪ Loss of opportunities of selecting new traits due to no artificial selection ▪ High carbon emission due to use of animal manure

factors which reduce the yield potential. In addition, low nitrogen supply to a crop causes poor grain quality which eventually results in poor baking quality due to the low protein content in grains. Also, under organic agriculture, the use of chemical fertilizers is restricted and nutrient supply is usually replaced by animal manure. However, recent researches indicate that the environmental effect on global warming potential (GWP) is higher in the organic agriculture than the conventional system. It results from the higher carbon emissions due to the manure transportation and application compared with the use of chemical fertilizers in the conventional system (Mohamad *et al.,* 2015). In terms of carbon-neutral crop (C emissions from agricultural practices - C sequestration from soil organic carbon), it is important for crops to mitigate carbon emissions and store carbon in soil (Monsanto, 2015) but a consistent application of animal manure can possibly lead to a higher carbon emission compared to conventional agriculture.

Wheat usually shows the best performance when weeds and diseases are highly restricted. Therefore, it is a challenge to select accessions which perform the best under low inputs. What is more, certain favorable traits which breeders want to select in a population could be ignored during natural selection. These conditions could inhibit elite accessions from showing up in a population (Phillips and Wolfe, 2005). In addition, uniform crop populations are usually preferable and also easier to manage by farmers. In heterogeneous crop populations, however, the commercial value could be diminished due to the low preference level by farmers. Also, less uniformity of crops will not satisfy the criteria of DUS (Distinct, Uniform and Stable) to obtain plant variety rights. In case of accessions under CCPs, it might be difficult to obtain plant variety rights. Therefore, for CCPs, it will be important to have a strategy that not only fortifies strengths and improves opportunities, but also to reduce weaknesses and eliminate threats by increasing favorable traits through consistent evolutionary breeding. That strategy will be a breakthrough in evolutionary breeding.

The objective of this research is, first of all, to evaluate yield and yield components of various winter wheat generations of CCPs with two different pure line varieties in order to elucidate its genetic performance. Second, to evaluate the vitality of wheat of CCPs compared to varieties in order to elucidate its competitive ability against poor conditions such as weeds. Finally, to investigate the adaptability and stability of CCPs compared with previous research data.

1.6. Research questions

Research questions to be answered consist of three main questions and each followed by three sub-questions.

1. In terms of genetic advance, does the genetic performance of CCPs increase over time?

1.1. Are there differences in yield and yield components between accessions?
1.2. Do CCPs increase yield and yield components over years under the low input farming systems?
1.3. Do the heritability increase over years under the CCPs?

2. In terms of vigor, does CCPs increase vitality of plants in the population?

2.1. Which CCP shows a good performance under the low input cropping system?
2.2. Do CCPs contain more favorable traits against weeds than varieties?
2.3. Do CCPs contain more favorable traits which affect early vigor than varieties?

3. In terms of stability, Is the performance of the CCPs stable over year?

3.1. Do CCPs show high adaptability in comparison with two pure line accessions?
3.2. Do CCPs exhibit better resistance against disease under low input cropping system?
3.3. Is the performance of the CCPs stable over years?

2. Material and Methods

2.1. Selection of parents

The winter wheat CCPs were created in the Organic Research Centre, Elm Farm in England. The parent accessions were selected in 2002 with respect to two target traits; high yield (Y) and high bread making quality (Q). In this respect, nine parents for high yield (Bezotaya^{1*}, Buchan^{2}, Claire^{3}, Deben^{4}, High tillering line^{5}, Norman^{6}, Option^{7}, Tanker^{8}, Wembley^{9}) and twelve parents for high quality (Bezostaya^{1*}, Cadenza^{2}, Hereward^{3}, Maris Widgeon^{4}, Mercia^{5}, Monopol^{6}, Pastiche^{7}, Renan^{8}, Renesansa^{9}, Soissons^{10}, Spark^{11}, Thatcher^{12}) were selected. One remarkable thing is that Bezostaya^{1*} was included in both groups and assumed only one trait was introduced to other accessions.

2.2. Creation of CCPs

Those twenty parents were crossed together in half-diallel to generate 190 F_1 cross combinations [n x (n-1) / 2, n=20] (Table 6) and the F_2 seeds were created by selfing from each of the individual F_1 plants. To ensure self-pollination, all ears of each F_1 were bagged. Each F_2 seed were bulked together respectively in order to create three different foundations of CCPs; $YCCP_n$, $QCCP_n$ and $YQCCP_n$. Therefore, each foundation CCPs is from 36 $YCCP_n$ identified as containing high yield potential (Y), 66 $QCCP_n$ with good baking quality and 88 $YQCCP_n$ generated from 190 crosses. Furthermore, in order to stimulate cross fertilization which results in high rates of recombination, male sterile CCP populations (CCP_{ms}) were also generated respectively to create foundation CCP_{ms}; $YCCP_{ms}$, $QCCP_{ms}$ and $YQCCP_{ms}$. Each foundation CCP_n or CCP_{ms} populations were intercrossed to create 1st CCPs. The cycling of CCP_n or CCP_{ms} was conducted in different areas in Europe (Döring *et al.,* 2015). This cycling project will allow evolutionary breeding of winter wheat to be sustained in various geographical locations. In addition, it will develop more robust accessions and biodiverse farming systems to overcome the weakness of conventional farming systems (Wolfe *et al.,* 2010). Wageningen University has been involved in this European cycling project since 2008. What is more, the new received populations have been re-sown every year in Wageningen to elucidate its progress in adaptability by evaluating the morphological and physiological traits which affects the yield potential (Nuijten and Lammerts van Bueren, 2013).

TABLE 6. Parental accessions and the possible combinations in half-diallel

		Buchan	**Claire**	**Deben**	**High tillering line**	**Norman**	**Option**	**Tanker**	**Wembley**	**Bezostaya**	**Cadenza**	**Hereward**	**Maris Widgeon**	**Mercia**	**Monopol**	**Pastiche**	**Renan**	**Renesansa**	**Soissons**	**Spark**	**Thatcher**
		Yield (Y)								**Bread making quality (Q)**											
Yield (Y)	**Buchan**		Y[1]	Y	Y	Y	Y	Y	Y	Y	YQ	YQ	YQ	YQ	YQ	YQ	YQ	YQ	YQ	YQ	YQ
	Claire			Y	Y	Y	Y	Y	Y	Y	YQ	YQ	YQ	YQ	YQ	YQ	YQ	YQ	YQ	YQ	YQ
	Deben				Y	Y	Y	Y	Y	Y	YQ	YQ	YQ	YQ	YQ	YQ	YQ	YQ	YQ	YQ	YQ
	High tillering line					Y	Y	Y	Y	Y	YQ	YQ	YQ	YQ	YQ	YQ	YQ	YQ	YQ	YQ	YQ
	Norman						Y	Y	Y	Y	YQ	YQ	YQ	YQ	YQ	YQ	YQ	YQ	YQ	YQ	YQ
	Option							Y	Y	Y	YQ	YQ	YQ	YQ	YQ	YQ	YQ	YQ	YQ	YQ	YQ
	Tanker								Y	Y	YQ	YQ	YQ	YQ	YQ	YQ	YQ	YQ	YQ	YQ	YQ
	Wembley									Y	YQ	YQ	YQ	YQ	YQ	YQ	YQ	YQ	YQ	YQ	YQ
Bread making quality (Q)	**Bezostaya**										Q	Q	Q	Q	Q	Q	Q	Q	Q	Q	Q
	Cadenza											Q	Q	Q	Q	Q	Q	Q	Q	Q	Q
	Hereward												Q	Q	Q	Q	Q	Q	Q	Q	Q
	Maris Widgeon													Q	Q	Q	Q	Q	Q	Q	Q
	Mercia														Q	Q	Q	Q	Q	Q	Q
	Monopol															Q	Q	Q	Q	Q	Q
	Pastiche																Q	Q	Q	Q	Q
	Renan																	Q	Q	Q	Q
	Renesansa																		Q	Q	Q
	Soissons																			Q	Q
	Spark																				Q
	Thatcher																				

[1]high yield (Y) potential (36), good baking quality (Q) potential (66), high yield + quality (YQ) potential (88)

2.3. Experimental materials

YQCCP$_n$ and YQCCP$_{ms}$ from Hungary have been grown, re-sown and harvested in bulk every year only based on natural selection at Droevendaal organic farm in Wageningen. In 2015, one YQCCP$_n$, eight YQCCP$_{ms}$ and two varieties *(Naturastar* and *Julius*) were sown as experimental materials (Table 7). Those two varieties were used as a reference to compare the performance of heterozygous CCPs. Barley was also sown as border crops along winter wheat to prevent cross fertilization. It is assumed that winter wheat containing the M.S trait lost genotype with male sterile (M.S) during several growing cycles among CCPs by continuous natural selection.

TABLE 7. Description of investigated composite cross populations

Genotype	Origin of the seeds	M.S	Cycling[1]
B	HU08-NL09-NL10-NL11-NL12-NL13-NL14-NL15	-	8th
A	HU08-NL09-NL10-NL11-NL12-NL13-NL14-NL15	$YQCCP_{ms}$	8th
G	HU09-NL10-NL11-NL12-NL13-NL14-NL15	$YQCCP_{ms}$	7th
D	HU10-NL11-NL12-NL13-NL14-NL15	$YQCCP_{ms}$	6th
E	HU11-NL12-NL13-NL14-NL15	$YQCCP_{ms}$	5th
H	HU12-NL13-NL14-NL15	$YQCCP_{ms}$	4th
I	HU13-NL14-NL15	$YQCCP_{ms}$	3rd
J	HU14-NL15	$YQCCP_{ms}$	2nd
L	HU15[2]	$YQCCP_{ms}$	1st
C	Naturastar (variety)	-	-
K	Julius (variety)	-	-

[1] Number of years grown in Wageningen (Droevendaal organic farm)
[2] New seed from Hungary

2.4. Experimental design

The experimental design was conducted at Droevendaal organic farm in Wageningen (Figure 9). Each plot was allocated through a randomized complete block design (RCBD) with three blocks. The total field size (96m x 24.5m) is composed of 11 plots within one block. Each plot (6m x 7.5m) is divided into 4 sub-plots with a width of 1.5m (Figure 10). All experiments were based

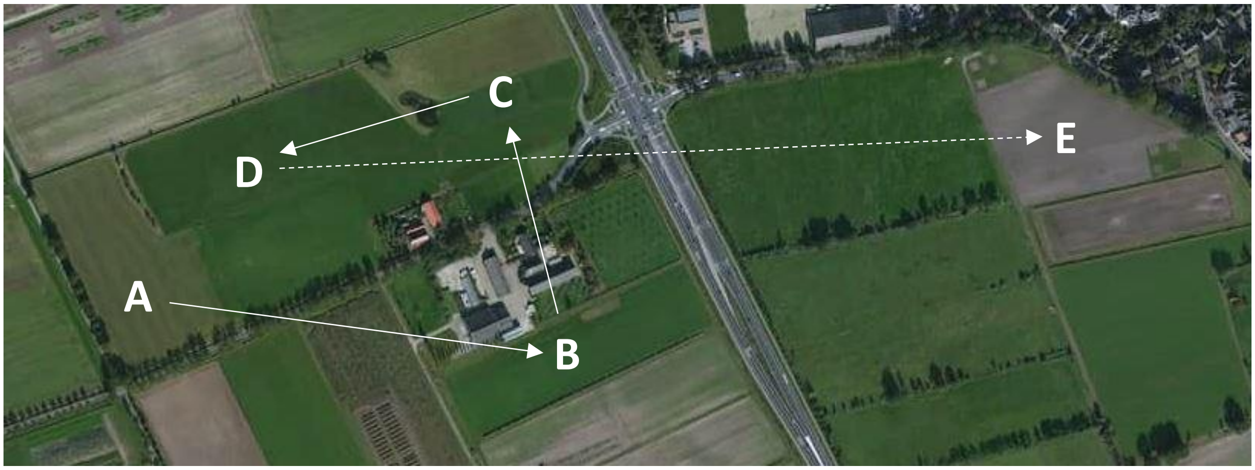


FIGURE 9. Experimental fields locations in *Droevendaal* organic farm in Wageningen, Netherlands. Field A, B, C and D indicate the location of the trials for prior 4 years; 2011-12, 2012-13, 2013-14 and 2014-15 respectively. The trial in 2015-16 was located in E

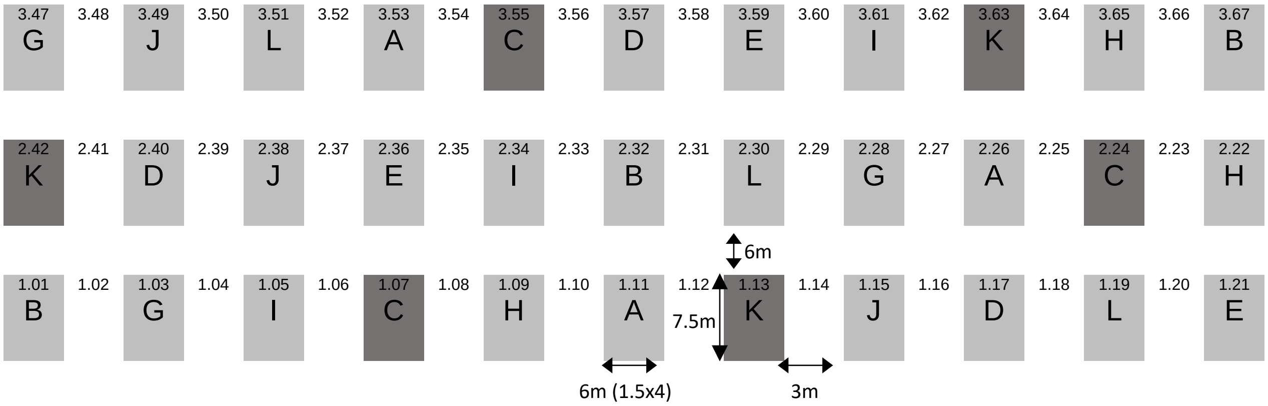
TABLE 8. Description of agronomic practices of organic field trials from 2008 to 2016

Year	Date (day/month/year)		N inputs (Kg·ha^{-1})	Crop rotation	No. of seed[1] (m^2)	*Remarkable notice*
	Sowing	Harvesting				
08~09	07/11/08	29/07/09	60	Phaseolus bean	200	
09~10	21/10/09	13/08/10	60	Oats/Faba bean	200	*Very cold spring and warm summer*
10~11	28/10/10	05/08/11	60	Spring Wheat	500	*Water lodging during winter and high weed infestation*
11~12	24/10/11	14/08/12	90	Spring barley	500	
12~13	24/10/12	14/08/13	90	Spring wheat	500	*Flooding in winter, high weed pressure due to poor germination (%)*
13~14	24/10/13	24/07/14	90	Spring wheat	500	*Yellow rust, partial flooding*
14~15	24/10/14	06/08/15	90	Spring wheat	500	*High weed infestation*
15~16	xx/10/16	24/08/16	90	-	500	*High weed infestation (Vicia sativa),*

[1] Number of sowing seeds in each growing season

on an organic farming system on sandy soil. Therefore, any mineral fertilizers and chemical treatment such as insecticide or herbicide were not considered. Before sowing, wheat seeds were treated by Tillecur from *Biofa AG* in Germany which is an organic mustard powder (1.5kg/100kg seed). All experimental materials were sown on October in 2015 according to the targeted seed density (200kg·ha^{-1}). The granule type of manure was applied with 90kg (N)·ha^{-1} on April 2016.

FIGURE 10. Experimental design 2015-2016 at Droevendaal organic farm in Wageningen



· Barley was sown between plots (white space) in the field (96m x 24.5m)
· Dark grey color indicates plots of varieties (Naturastar and Julius)

2.5. Sample size and sampling method

To decide the number of samplings, *G*POWER* data analysis was conducted. The raw data from 2014/2015 of the 6 different varieties was measured in order to determine the sample size. Those data were inputted into the *G*POWER* software program and analyzed (under α=0.05, 1-β=0.95). The sample size of each variate was calculated based on the statistical power (1-β) and each variate showed a different sample size under the (1-β) probability (Figure 11).

In this research, a 95% statistical power (1-β) regarding samplings was suggested. On the whole, all variates were satisfied with 95% power (1-β) when the total sample size was more than 550. Therefore, 50 samplings per plot (50 x 11 plot = 550) was decided. In the experimental design, one plot was divided into 4 sub plots. So, 15 plants from sub-plot of both ends and 10 plants from the two middles were randomly selected. Each selected plant was tagged with a red ribbon for further measurements. Finally, the selection of plants at the edge of each sub-plot was ruled out in order to avoid border effect and only plants inside the rows were selected for further assessments.

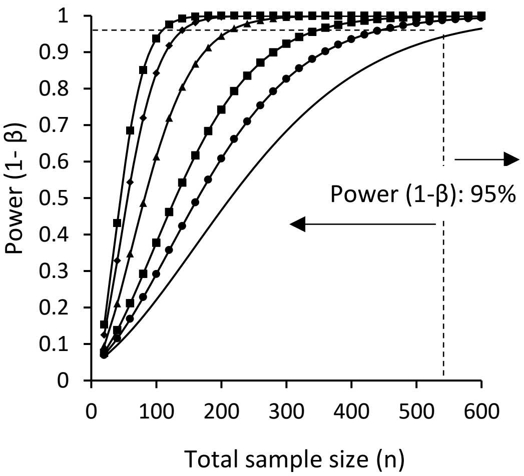


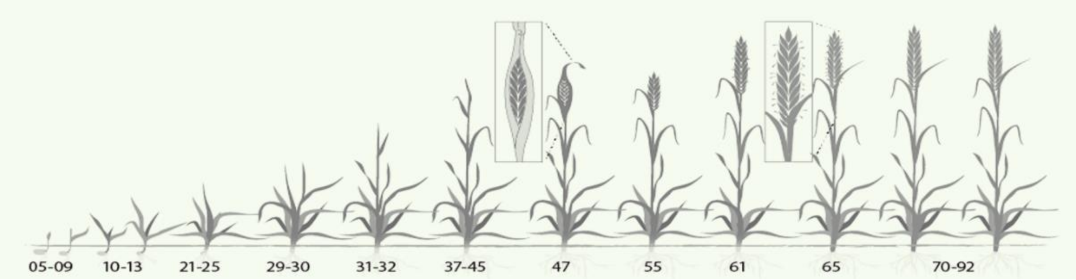
FIGURE 11. The sample size under the probability of power (1- β). Six variates (plant height, flag leaf and spike length, number of spikelet, fertility of ear and distance between flag leaf and spike) were selected for determining the sample size.

2.6. Measurements

From seeds to harvested wheat, all growing stages are required to be measured and assessed in order to evaluate its overall performance. Some physiological and morphological traits are measured phenologically during growing stages in the field. Final yield (ton·ha^{-1}) and thousand kernel weight (TKW) were measured in the Unifarm, Wageningen University after seed cleaning according to the protocol of the Unifarm in Wageningen University.

TABLE 9. Description of BBCH-scale of wheat (BBCH Scale, 2010)

Code	Description
	Growth stage 0: Germination
00 ~ 09	· Beginning of seed imbibition ~ Emergence
	Growth stage 1: Leaf development
10	· First leaf through coleoptile
11 ~ 19	· First leaf unfolded ~ 9th or more leaves unfolded
	Growth stage 2: Tillering
20	· No tillers
21	· Beginning of tillering: first tiller detectable
22 ~ 29	· 2nd tillers detectable ~ 8th tillers detectable or Maximum number of tillers detectable
	Growth stage 3: Stem elongation
30	· Beginning of stem elongation: pseudostem and tillers erect, first internode begins to elongate, top of inflorescence at least 1 cm above tillering node
31	· First node at least 1 cm above tillering node
32 ~ 36	· Node 2 at least 2 cm above node 1 ~ Node 5 at least 5 cm above node 1
37	· Flag leaf just visible, still rolled
39	· Flag leaf stage: flag leaf fully unrolled, ligule just visible
	Growth stage 4: Booting
41	· Early boot stage: flag leaf sheath extending
43	· Mid boot stage: flag leaf sheath just visibly swollen
45	· Late boot stage: flag leaf sheath swollen
47	· Flag leaf sheath opening
49	· First awns visible (in awned forms only)
	Growth stage 5: Inflorescence emergence, heading
51	· Beginning of heading: tip of inflorescence emerged from sheath, first spikelet just visible
52 ~ 59	· 20% of inflorescence emerged ~ End of heading: inflorescence fully emerged
	Growth stage 6: Flowering, anthesis
61 ~	· Beginning of flowering: first anthers visible
65	· Full flowering: 50% of anthers mature
69	· End of flowering: all spikelets have completed flowering but some dehydrated anthers may remain
	Growth stage 7: Development of fruit
71 ~ 77	· Watery ripe: first grains have reached half their final size ~ late milk
	Growth stage 8: Ripening
83 ~ 89	· Early dough ~ Fully ripe: grain hard, difficult to divide with thumbnail
	Growth stage 9: Senescence
92 ~ 99	· Over-ripe: grain very hard, cannot be dented by thumbnail ~ Harvested product



(http://www.nordiskalkali.se/strasad)

2.6.1. BBCH-scale

The BBCH-scale is a method to determine the developmental stage of wheat. It provides a code number and description according to each growth stage (Table 9). This method is used as a common language regarding the developmental stage of wheat.

2.6.2. Early seedling emergence

The early number of plants per m^2 was counted during winter, using a square iron frame (45 x 45cm). This counting was conducted per each sub-plot. Therefore, 4 replications were conducted on each plot.

2.6.3. Seed germination test

Seed vigor was examined by the rate of germination per each accession harvested in growing season 2014/15 and new CCP from Hungary (HU15). The germination test was conducted according to the ISTA regulation. The Between paper (BP) method, in which the seeds are germinated between two layers of paper was used as growing media. 150 seeds were selected from the representative sample. 50 seeds were placed on the BP which is supposed to be rolled with another paper. 3 replicates per a cultivar were placed in the chamber at 20°C, 65% humidity for 8 days. No treatments to break the dormancy were conducted in this germination test. On day 4, the first count was conducted and final count was on day 8. Seedling evaluation was based on the rules of ISTA.

2.6.4. Number of tillers

The number of tillers per m^2 was investigated both in early and late spring, using a square iron frame (45 x 45cm). The number of tillers was counted at the tillering stage (BBCH-scale: 21 to 29) in order to analyze whether or not early tillering affects any crop performances. In addition, fertile tillers which have a spike were also counted at the flowering and anthesis stages (BBCH-scale: 61 to 69) in order to investigate tillering capacity. The counting was conducted on each sub-plot. Therefore, 4 replications were conducted on each plot.

2.6.5. Ground cover rate

The ground cover rate was measured during the tillering stage of the wheat (BBCH-scale: 20 to 29) by image analysis, with the help of the MATLAB software program. In order to extract the green color on each image, the CIVE (Color Index of Vegetation Extraction) program was designed in MATLAB. The picture was taken 2 times on each sub-plot. Therefore, in case of image analysis, 8 replications were conducted on each plot.

2.6.6. Weed density

The weed density per m^2 was investigated at the stem elongation stage (BBCH-scale: 30 to 39), using a square latticed frame (75 x 75cm) which has 100 holes. If the weeds were filled with a hole, it was counted as 1 %. Weed density was investigated per each sub-plot. Therefore, 4 replications were conducted on each plot.

2.6.7. Flag leaf and greenness measurement

The width and length of flag leaves were measured from 50 randomly selected plants per plot when the flag leaves were fully unrolled and when the ligule was visible. The area of the flag leaves was calculated using the following formula (Stickler *et al.,* 1961);

- Leaf area (cm^2) = L x W x F

  [*L = Maximum length (cm), W= Maximum width (cm), F= factor 0.707 for wheat*]

The greenness of the flag leaf was measured to indicates nutrient efficiency and potential grain quality. In order to investigate the greenness of the leaf, 50 leaves per plot were randomly collected and a picture of each leaf was taken respectively. Therefore, the image analysis of 150 pictures per each cultivar was processed by a web-based freeware software. Each color has a specific 6-digit color code known as the *hex color code*. On the basis of the color code, different color percentage was calculated from the picture and only the green color was extracted (Figure 12).

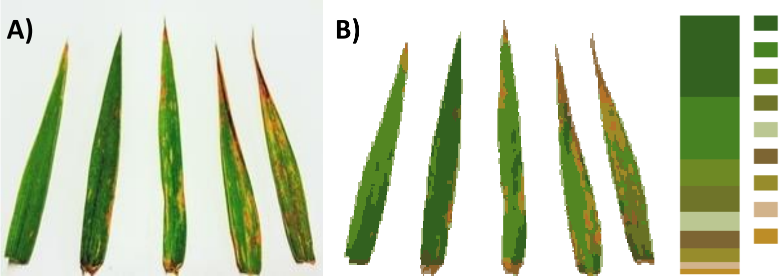


FIGURE 12. The percentage of greenness of flag leaf by image analysis. A) Flag leaf pictures B) The Image analysis by web-based software program (http://www.coolphptools.com) and each color was extracted from the image and color percentage was calculated automatically using hex color code.

2.6.8. Flowering time

The initial and terminal date of the flowering time was recorded in each plot and compared with previous growing seasons in order to elucidate different flowering times over years.

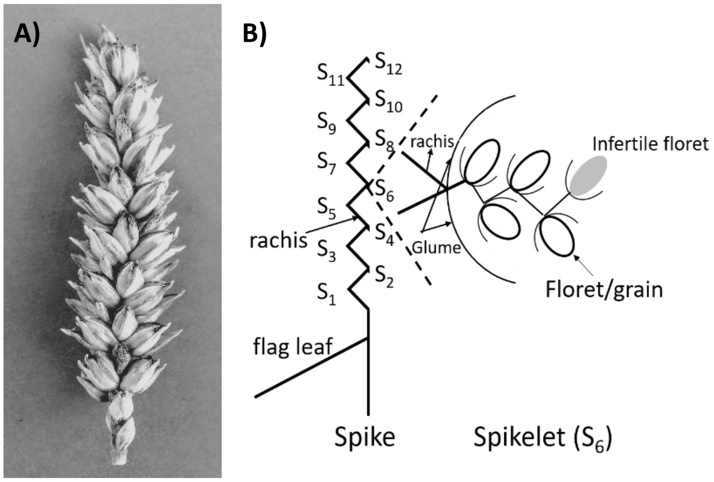


FIGURE 13. The structure of spike. A) one spike consists of several spikelets B) one spikelet consists of several florets.

2.6.9. Spike fertility

Spikelets containing a milky grain were considered as a fertile spikelet at the fruit development stage (BBCH-scale: 71 to 79). On the contrary, spikelets that failed to form a grain were counted as infertile spikelet (Figure 13). In order to investigate the spike fertility, the total number of fertile and infertile spikelets per spike were counted from 50 randomly selected plants per plot. The spike fertility (%) for each cultivar can be calculated using following formula:

- $\text{Spike fertility (\%)} = \dfrac{\text{Number of fertile spikelet per a spike}}{\text{Number of total spikelet per a spike}} \times 100$

2.6.10. Spike length and Spikelet density

The spike length was measured from the bottom to the top of the spike, excluding the awns from 50 randomly selected plants per plot. The distance between the spikelet within a spike can be defined as the spikelet density (%) and can be calculated using the following formula:

$$\text{▪ Spikelet density (\%)} = 1 - \frac{\text{Spike length (cm)}}{\text{Total number of spikelet per a spike}} \times 100$$

2.6.11. Plant height

The plant height was measured from 50 randomly selected plants per plot during the ripening stage (BBCH-scale: 83 to 89). The plant height was measured from the ground to the top of the spike, excluding the awns.

2.6.12. Distance between the flag leaf and spike

The distance between the flag leaf and the base of the spike was measured from 50 randomly selected plants per plot during the senescence stage (BBCH-scale: 92 to 99)

2.7. Plot damage

In this research, the experimental field is composed of 132 sub-plots. It indicates that each sub-plot might be respectively affected by various external conditions. Therefore, the plot damages caused by different factors were measured per each sub-plot before harvesting. The harvested yield in each sub-plot was adjusted according to the plot damage (%).

$$\begin{array}{c}\text{Adjusted yield}\\ (\text{ton}\cdot\text{ha}^{-1})\end{array} = \text{harvested yield} + (\text{harvested yield}) \times \frac{\text{damaged area (m}^2\text{) of a sub-plot}}{\text{total area (m}^2\text{) of a sub-plot}}$$

Each sub-plot damage was observed the day before harvesting to compensate the yield loss. Two types of a different damage, mechanical and weed damage were generally observed (Table 10). Mechanical damage is presumably caused by tractor movement and was shown since early seedling stage. In the second experimental block, *Julius (K)* showed the most severe mechanical damage since the early seedling stage. The young wheat seedlings were not able to recover from the damage and finally the plot was ruled out from the experiment. Therefore, in this research, *Julius (K)* had only two replicates (blocks). Weed damage was usually caused by *Vicia sativa* which suppressed wheat growth. Weed damage usually occurred at the edge of plots as weed control was consistently conducted inside the plots whereas weeds in the border crop (i.e. barley) were not actively removed. So, the *Vicia sativa* grown outside plots continuously moved toward experimental plots and negatively affected wheat growth.

TABLE 10. Summary of plot damage (%) in growing season 2015/2016

Cycling year	Acce-ssions	Mechanical damage (%)				Weed damage (%)				Overall Mean (%)
		Block 1	Block 2	Block 3	Mean	Block 1	Block 2	Block 3	Mean	
9	B	-	0.7	14.7	7.7	6.3	3.0	-	4.7	4.1
9	A	-	2.0	4.7	3.4	-	3.5	-	3.5	1.7
8	G	-	-	27.7	27.7	-	3.0	-	3.0	5.1
7	D	-	13.3	1.3	7.3	3.4	-	-	3.4	3.0
6	E	-	3.6	5.2	4.4	9.3	3.3	-	6.3	3.6
5	H	-	8.0	12.0	10.0	0.5	1.3	-	0.9	3.6
4	I	-	1.5	14.7	8.1	3.5	-	-	3.5	3.3
3	J	-	-	20.7	20.7	2.8	4.1	-	3.5	4.6
2	L	-	-	10.7	10.7	7.1	13.7	-	10.4	5.2
-	C	-	7.0	11.6	9.3	2.3	5.9	-	4.1	4.5
-	K	-	100.0[1]	29.0	64.5	1.5	-	-	1.5	21.7

[1] Fully damaged (discarded from the experiment)

2.7.1. Weed infestation

In order to exhibit the degree of weed infestation in the whole field, the dark green color was illustrated as the high weed population and the light green was indicated as the middle weed population, while the grey color indicates the low population of weed in the field (Figure 15). Block 2 showed the most severe weed infestation while block 3 exhibits the least weed populations. Cultivar K in block 2 was ruled out due to growth failure in the field. Weed growth is usually ceased at a low temperature. In this growing season, it was observed that weeds grew abruptly since early May. Based on the weather record in Wageningen, the Netherlands, the temperature around early May had risen to 20°C (Figure 14). It is well known that the emergence of weeds is related to several environmental factors such as soil conditions temperature, water and light. A previous research (Calado *et al.,* 2009) indicates the relationship between the sum of the daily mean temperature and the percentage of weed emergence. According to the result, weed emergence increased rapidly when the accumulated temperature was reached within a certain period of time. Therefore, it can be inferred that weeds could be rapidly grown at an accumulated temperature of higher than 20°C for a certain period of time.

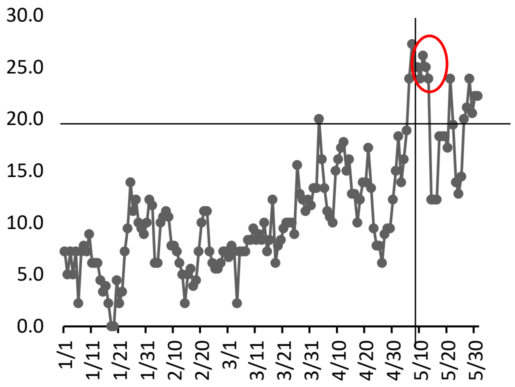
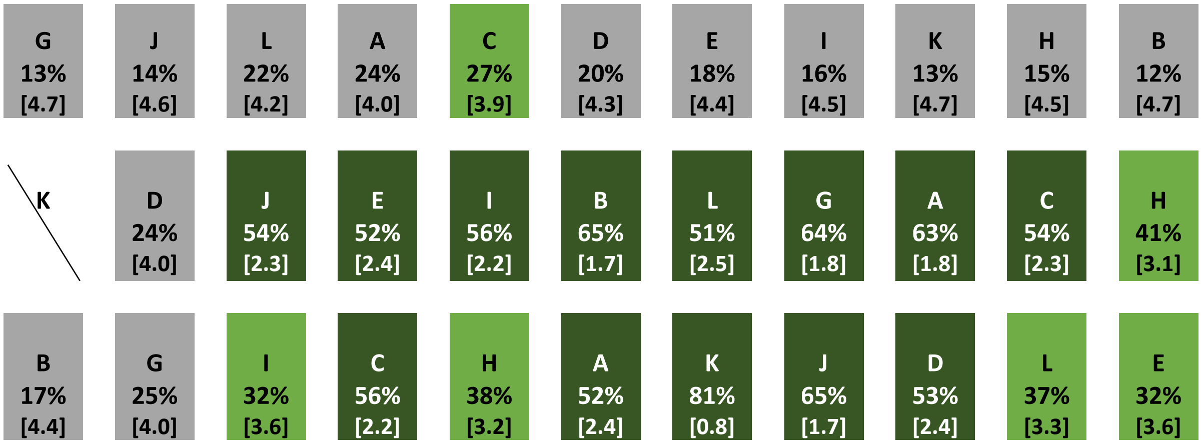


FIGURE 14. The weather records in Wageningen area from January to May in 2016. (x-axis: date(mm/dd), y-axis: temp., °C) (www.weather.com)

FIGURE 15. **The degree of weed infestation in each plot among blocks**



G 13% [4.7]	J 14% [4.6]	L 22% [4.2]	A 24% [4.0]	C 27% [3.9]	D 20% [4.3]	E 18% [4.4]	I 16% [4.5]	K 13% [4.7]	H 15% [4.5]	B 12% [4.7]
K	D 24% [4.0]	J 54% [2.3]	E 52% [2.4]	I 56% [2.2]	B 65% [1.7]	L 51% [2.5]	G 64% [1.8]	A 63% [1.8]	C 54% [2.3]	H 41% [3.1]
B 17% [4.4]	G 25% [4.0]	I 32% [3.6]	C 56% [2.2]	H 38% [3.2]	A 52% [2.4]	K 81% [0.8]	J 65% [1.7]	D 53% [2.4]	L 37% [3.3]	E 32% [3.6]

† Weed density (%) per m^2

†† Scores: [1] fully covered weeds [2] most covered weeds [3] few covered weeds [4] few weeds, not covered ground [5] no weeds.

††† Mean of each accession: A [61.6%], **B [41.6%]**, **C [60.7%]**, D [43.2%], E [45.5%], G [45.5%], **H [41.8%]**, I [46.3%], J [58.8%], K [62.8%], L [48.9%]

Although weeds were continuously removed, new weeds emerged again. Vetches (*Vicia sativa*) were predominant in many plots but also other weeds appeared in the field (Figure 16). While most weeds were easy to control both at the early and late stage, in case of vetches, however, it was difficult to be manage when it was fully grown due to its twisted branches that cover the wheats completely. As a consequence, wheats got tangled with vetches and could not fully grow as the vetches suppressed the wheats. Also, while eliminating vetches, spikes were damaged as many vetches' branches were tangled with spikes. In this research, *Vicia sativa* was the most serious weed problem which results in yield loss in organic farming systems. Therefore, to understand the physiological and morphological characteristics of each weed and to prioritize which weed species are controlled first before rapid growth might be a crucial procedure in low-input farming systems.

FIGURE 16. **The main weed species grown in wheat field.** (A) Hairy Vetch (B) Clover (C) Flixweed (D) Fat Hen (E) Horseweed (F) Cornflower. (Pictures were taken on 09th June 2016, in Droevendaal organic farm)

2.8. Harvesting

Harvest for all plants in the field was conducted according to the protocol of *Unifarm* in *Wageningen University*. Each sub-plot was harvested separately. After harvesting, seed cleaning was conducted and the total yield ($ton \cdot ha^{-1}$) was measured.

2.8.1. Thousand-kernel weight (TKW)

After the seed cleaning, 1,000 seeds per each sub-plot were selected and the TKW (g) per each sub-plot was measured. Therefore, 12 replicates for the TKW were conducted. All data was analyzed as ANOVA analysis to compare its significant differences among accessions.

2.9. Statistical analysis

The analysis of the variance and regression model were mainly performed with the help of the GENSTAT software program. For determining the significant sample size, the *G*POWER* software program was used. All statistical analysis was conducted under the 5% level of significance ($\alpha=0.05$).

2.9.1. Analysis of background data

Data comparisons from the 2012 to 2016 growing seasons were conducted to elucidate how CCPs changed over the years. There were two types of data analyzed. The plot mean data was analyzed for early vegetative traits such as tillering and the ground cover rate, which were investigated per plot, not a plant (Appendix 1). On the contrary, raw data was analyzed for the plant height, flag leaf and yield components which were investigated per plant (Appendix 2, Appendix 3). Also, yield ($ton \cdot ha^{-1}$) and TKW (g) were analyzed by raw data (Appendix 4). Unlike inbred or hybrid cultivars, CCPs are genetically diverse. Therefore, traits based on individual plants were analyzed by raw data because analysis of the plot mean data might distort statistical outputs such as the p-value or LSD.

2.9.2. Finlay-Wilkinson Regression model

In order to evaluate adaptability of each accession, the Finlay-Wilkinson regression was suggested. In this research, each growing season was considered as an environmental index and the linear regression model was applied to calculate the adaptability. In terms of $V_P = V_G + \beta_E + e$, the performance and adaptability of each accession was analyzed.

2.9.3. ANOVA analysis

The effect of genotype with blocks was analyzed by one-way ANOVA analysis with one factor; genotype. Also, as multiple comparisons with genotypes, the least significant differences (LSD) test was calculated to elucidate the differences regarding each variate (y) among the accessions. Also in order to elucidate the G x E interaction, two-way ANOVA was conducted with two factors; genotype and year.

2.9.4. Non-parametric data analysis

The normality test was conducted in all investigated data of each trait (Appendix). In case, the data did not follow the normality, the LSD test was replaced by the *Bonferroni correction* in order to conduct more accurate multiple comparisons among genotypes. When the normality test was conducted, the data of *Julius (K)* was excluded because in this season, the *Julius (K)* almost failed to grow and non-parametric data pattern might be highly caused due to data outliers,

2.9.5. Coefficient of variance (CV)

Each variate (y) has a vast difference of measured value according to its size or measuring method. Therefore, the coefficient of variance (CV) was calculated relatively to evaluate the extent of distribution of the measured value from the grand mean. In case of the CV, to avoid distortion of the standard deviation, raw data was used to calculate the CV (%) instead of plot mean data.

- $CV = \frac{\sqrt{V}}{\bar{x}}$, *Where V= variance,* $\bar{x}$*= grand mean of measured value*

2.9.6. Heritability

As assumed by the ANOVA analysis, the heritability was calculated by applying expected mean squares (EMS). When different genotypes (n) were cultivated with replicates (r) with one factor, the following formula for heritability was applied.

Source of Variation	**df**	**M.S (σ^2)**	**EMS**
Genotype	n-1	MS_G (σ_G^2)	$\sigma_E^2 + r\cdot\sigma_G^2$
Block	r-1	MS_B (σ_B^2)	$\sigma_E^2 + n\cdot\sigma_B^2$
Error	(n-1)(r-1)	MS_E (σ_E^2)	σ_E^2
Total	nr-1		

$$\text{Heritability} = \frac{\text{genotypic var.}(\sigma_G^2)}{\text{phenotypic var.}(\sigma_P^2)} = \frac{\sigma_G^2}{\sigma_G^2 + \sigma_E^2} \quad ;\text{ where } \sigma_G^2 = \frac{\sigma_G^2 - \sigma_E^2}{r}$$

2.9.7. Shannon index

For the quantitative measure, the Shannon index was applied as a diversity index to evaluate the difference among accessions using the following formula (Shannon and Weaver, 1949).

$$H' = -\sum_{i=1}^{r} Pi \ \mathrm{In} \ \mathrm{P}i$$

Where Pi = the proportion of total number of investigated individuals in the i^{th} class, r = the number of phenotypic classes for a trait

3. Results

The cultivation report during the 2015/2016 growing season is summarized in Table 11. Research data of the investigated agronomic traits of the different accessions in 2015/2016 are arranged from the oldest CCP at the top to the newest towards the bottom. Two varieties, *Naturastar (C)* and *Julius (K)* were arranged at the end of table.

TABLE 11. Cultivation report of wheat production in growing season 2015/2016

Growth stage		BBCH	Starting date[1] (d/m/y)	Remarkable notice
1	Leaf development	10 ~	2015 ~	▪ Mechanical damage in block 3 and Julius of block 2
2	Tillering	20 ~	14/03/16	▪ Continued raining / cloudy weather ▪ Harrowing (06/04/16) ▪ Manure applied (20/04/16)
3	Beginning of stem elongation	30 ~	02/05/16	▪ Sunny day for a while since 05/05/16 ▪ Harrowing (03/05/16)
4	Booting	41 ~	06/05/16~	▪ Rapid weeds growth
	First awns visible	49 ~	13/05/16	▪ Julius discarded in block 2
5	Beginning of heading	51 ~	23/05/16	▪ Ear damages by rabbits
6	Beginning of flowering	61 ~	28/05/16	▪ High weed infestation (*Vicia sativa*)
	Full flowering (50%)	65 ~	10/06/16	
	End of flowering (100%)	69 ~	14/06/16	
7	Development of fruit	71 ~ 77	20/06/16	▪ Barley adjacent wheat was harvested except *border crops* between plots (06/07/16)
9	Harvesting		24/08/16	▪ Harvested per sub-plot
	Seed cleaning		05/09/16~	▪ Measuring TKW

[1] based on CCPs

TABLE 12. The summary table for early vegetative traits in growing season 2015/2016

				Number of tillers (m^2)						Ground cover rate							
	Seedling[2] (m^2)			Early stage			Late stage			Early stage				Late stage		Weed density	
	Mean (#)		CV (%)	Mean (#)		CV (%)	Mean (#)		CV (%)	Mean (%)		CV (%)	score	Mean (%)	CV (%)	Mean (%)	CV (%)
B (9)[1]	358	b[3]	8.5	609	b	11.9	359	b	15.9	25.1	b	13.5	4.0[4]	37.2	10.2	41.6	83.4
A (9)	351	b	6.2	611	b	12.9	359	b	12.2	27.0	b	10.1	4.3	36.0	5.9	61.6	39.6
G (8)	340	b	6.9	547	ab	16.0	347	ab	13.3	24.5	ab	15.9	3.8	37.2	7.6	45.5	68.1
D (7)	367	b	7.1	571	b	7.6	366	b	16.5	25.8	b	16.2	4.1	37.2	10.1	43.2	49.3
E (6)	357	b	4.7	584	b	15.1	342	ab	15.1	25.6	b	27.4	4.1	35.0	19.1	45.5	43.7
H (5)	366	b	6.1	585	b	7.5	376	b	11.5	26.4	b	15.5	4.2	37.3	10.6	41.8	41.2
I (4)	349	b	7.2	596	b	18.7	353	b	14.4	27.4	b	13.0	4.4	36.1	7.9	46.3	55.2
J (3)	351	b	10.0	589	b	17.1	347	ab	23.1	26.9	b	16.5	4.3	37.5	6.5	58.8	58.4
L (2)	332	b	8.1	584	b	16.1	306	ab	9.4	24.4	ab	21.3	3.8	34.1	14.4	48.9	37.8
C	366	b	5.9	460	ab	12.8	322	ab	17.7	21.4	ab	16.5	3.2	32.3	10.1	60.7	32.5
K	270	a	7.0	370	a	23.3	264	a	13.6	16.0	a	23.6	2.1	28.1	25.1	62.8	78.2
F-^{value}	9.0			4.11			3.50			3.69				1.61		0.94	
P-^{value}	<0.001			0.003			0.009			0.006				0.175		0.520	
LSD	27.15			107.83			47.68			0.051				-		-	

[1] (number) indicates CCPs cycling years

[2] Number of early seedling in Droevendaal organic farm

[3] Different letters within on characteristic indicate a significant difference (P<0.05) according to the LSD test

[4] Ground cover rate score: 1) very poor 2) poor 3) fair 4) good 5) very good

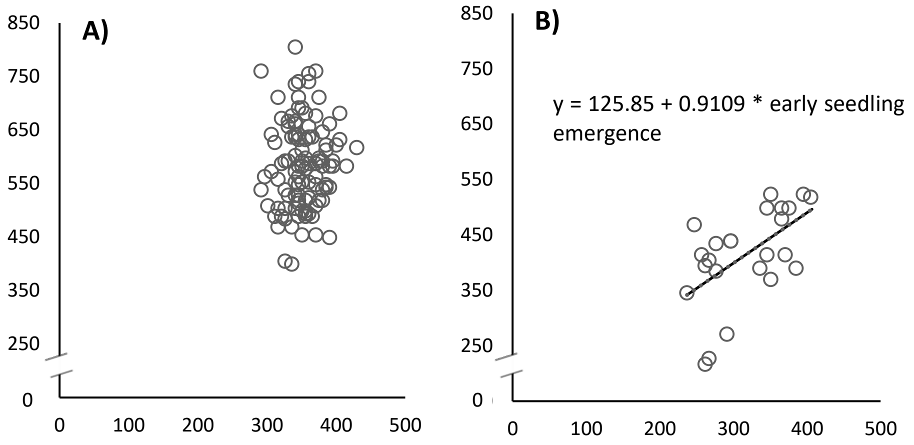


FIGURE 17. The linear regression model between early seedling emergence (x-axis) and tillering (y-axis). A) No regression was shown in CCPs B) Varieties show regression model between early seedling numbers and tillering

3.1. Early vegetative traits

The result of the early vegetative traits is summarized in Table 12. Early seedling emergence showed a significant difference (p<0.001) among accessions. *Julius (K)* showed the lowest seedling emergence, while all CCPs and *Naturastar (C)* showed the same seedling emergence. There was no regression (p=0.512) between early seedling emergence and tillering among CCPs (Figure 17 A), while two varieties showed a regression (p=0.005) between two parameters (Figure 17 B).

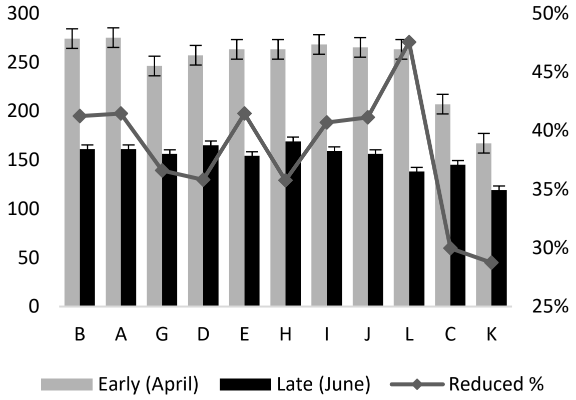


FIGURE 18. The number of tillers at early and late stage (BBCH-scale: 20 to 29). Left y-asix indicates number of early tillers and right y-asix indicates reduced rate (%) of tillers between early and late tillering stage. † Reduced rate (%) of tillers: (early number of tillers – late number of tillers) / early number of tillers.

The number of tillers also showed a significant difference (p<0.05) among accessions. *Julius (K)* showed the lowest number of tillers both during early and late tillering stage. However, compared with the reduced rate (%) of tillers between early (April, 2016) and late (June, 2016) tillering stage, *Naturastar (C)* and *Julius (K)* showed the lowest reduced rate (%) in comparison with CCPs. On the contrary, *CCP-L* showed the highest reduced rate (%) of tillering (Figure 18).

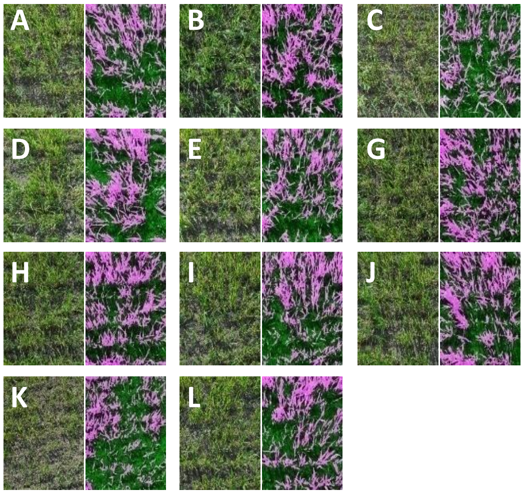


FIGURE 20. Images were processed and only green color was extracted (pink color) from each image with the method of CIVE (Color Index of Vegetation Extraction). The area of green color was automatically calculated by MATLAB.

The ground cover rate was calculated both during the early and late tillering stage (BBCH-scale: 20 to 29) by the image analysis in MATLAB (Figure 20). Also, the visual observation with respect to the ground cover rate was performed as scoring [1 to 5] in order to obtain both qualitative and quantitative data of the ground cover rate. Those two sets of values were analyzed as a regression model (data was not shown) to calculate a proportional relationship between two different data. Therefore, each quantitative value of the ground cover rate calculated by MATLAB has its corresponding qualitative observation score (Table 12). There was a significant difference (p=0.006) in the ground cover rate during the early stage among accessions. However, no significant difference (p=0.175) was shown during late stage. The lowest ground cover rate was shown in *Julius (K)* while CCPs and *Naturastar (C)* showed similar ground cover rate at early stage. However, just because the ground cover rate was high, it did not mean that it shows a higher suppressing capacity against weeds. The weed density did not show a regression model (p=0.322) between the ground cover rate and the weed populations (data was not shown). Also, the weed density did not show a significant difference (p=0.520) among accessions. In the experimental field, vetches (*Vicia sativa*) were the most predominant weed that covered the wheats completely (Figure 19). Although other weed species were also observed in the field, those were not highly visible within the plots as the wheats were taller than other weed species except for the vetches. Therefore, it can be suggested that weed populations can be more affected by the morphological traits of weeds, not the number of weeds. Furthermore, in this growing season, the plant growth habit among accessions were not distinct. Also, the CV (%) of the weed density was much higher than other early vegetative traits. It means that, in this growing season, vetches were widely distributed by many branches and no other distinct traits were found against weed density.

FIGURE 19. The growth of vetches (*Vicia sativa*) in organic wheat field. *Vicia* covered wheats completely and hindered wheat growth. † The picture was taken on 06th, June 2016, in Droevendaal organic farm.

TABLE 13. **The summary tables for late vegetative traits in growing season 2015/2016**

				Flag leaf								Distance between flag leaf and spike			Disease symptoms					
	Plant height			Length			Width			Area					Leaf[3]			Spike		
	Mean (cm)		CV (%)	Mean (cm)		CV (%)	Mean (mm)		CV (%)	Mean (cm^2)	CV (%)	Mean (cm)		CV (%)	Mean (%)		CV (%)	Mean (%)		CV (%)
B (9)[1]	86.1	e[2]	8.1	12.0	ab	16.3	10.5	bc	13.0	9.0	24.8	16.4	cd	0.19	18.7	b	27.0	3.2	ab	63.0
A (9)	83.0	cd	8.4	11.5	a	20.0	10.3	ab	14.2	8.5	29.1	15.9	bc	0.26	20.7	b	20.1	3.5	ab	41.5
G (8)	81.6	c	10.5	11.7	ab	18.5	10.7	cd	13.6	8.9	27.0	15.3	b	0.24	14.0	ab	14.3	3.4	ab	42.3
D (7)	85.8	e	8.2	11.9	ab	17.9	10.4	bc	13.3	8.8	26.2	16.9	d	0.03	18.0	ab	22.2	4.7	b	32.8
E (6)	84.5	de	9.8	11.6	ab	19.0	10.7	cd	13.5	8.9	28.7	17.9	e	0.06	16.7	ab	13.9	3.8	ab	43.0
H (5)	81.6	c	7.9	12.1	ab	18.1	10.1	a	12.1	8.8	27.4	15.1	b	0.15	17.3	ab	17.6	5.0	b	86.6
I (4)	83.4	cd	9.0	12.0	ab	17.3	10.4	abc	13.2	8.9	25.0	15.9	bc	0.04	13.3	ab	43.3	3.3	ab	49.7
J (3)	84.7	de	10.6	12.4	b	18.1	10.4	abc	14.1	9.2	27.9	17.0	de	0.36	15.3	ab	32.8	4.5	ab	32.4
L (2)	84.4	de	8.4	12.0	ab	18.0	10.4	abc	12.4	8.9	25.2	16.3	cd	0.27	14.7	ab	28.4	3.2	ab	58.4
C	78.4	b	6.3	12.2	ab	19.5	10.5	bc	11.8	9.1	27.3	15.7	bc	0.10	12.7	ab	32.9	8.5	c	82.6
K	66.0	a	9.5	11.5	a	19.7	11.1	d	14.9	9.2	30.0	11.0	a	0.18	8.0	a	35.4	2.6	a	49.7
F-value	136.79			2.67			3.47			1.08		28.06			2.79			4.07		
P-value	*<0.001*			*0.003*			*<0.001*			*0.374*		*<.001*			*0.022*			*<0.001*		
LSD	1.309			0.48			0.2985			-		0.92			0.0715			0.0229		

[1] (number) indicates CCPs cycling years

[2] Different letters within on characteristic indicate a significant difference (P<0.05) according to the LSD test

[3] Leaf disease = Septoria tritici blotch + powdery mildew

3.2. Late vegetative traits

The result of the late vegetative traits was summarized in Table 13. The plant height showed a significant difference (p<0.001) among accessions. *CCPs- B* showed the highest height while the lowest height was shown by *Julius (K)*. There was a positive regression in accessions between the ground cover rate and plant height except for *CCPs- H* and *Julius (K)* (Figure 21 A). On the other hand, a negative regression model was shown between the weed density and plant height (Figure 21 B). The flag leaf area and plant height were also analyzed to elucidate a regression model. All accessions showed a positive regression except for *CCPs- H*

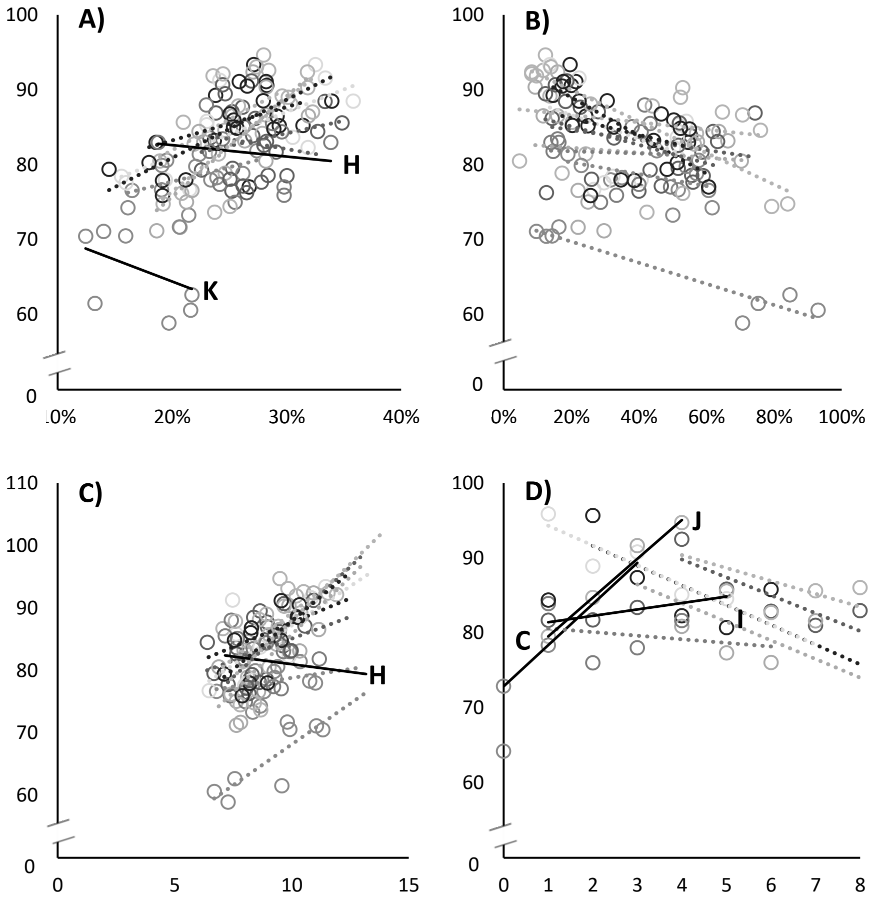


FIGURE 21. The linear regression model of plant height. A) Early ground cover rate and plant height and B) Weed density and plant height C) Flag leaf area and plant height D) The number of CCPs cyclings and plant height (Linear forecast was applied to the regression model of CCPs cyclings as each CCPs cultivars have different cycling years).

(Figure 21 C). However, there was no regression between tillering and plant height in CCPs. Therefore, as a multiple regression model; $y_i = \beta_0 + \beta_1 x_{1i} - \beta_2 x_{2i} + \beta_3 x_{3i} + \varepsilon_i$, the overall plant height (y_i) of CCPs can be estimated by;

> $y_i = 61.47 + 41.85\, x_{1i} - 5.54\, x_{2i} + 1.54\, x_{3i} + \varepsilon_i$, for i = 1,…9 with $\varepsilon_i \sim N(0,\sigma)$, independent from each other (where y_i=plant height, x_1=ground cover rate, x_2=weed density, x_3=flag leaf area)

Furthermore, data of previous growing seasons were analyzed in order to show the trend of plant heights over years. The plant height decreased over CCPs cyclings, except for the *CCPs- I* and *CCP-J* (Figure 21 D). However, *CCPs- I* did not show a significant difference over the years (Table 14). Also, *CCP- J* only had three times of CCPs cycling. Therefore, more time might be needed to analyze the plant height trend of CCPs- I and CCP-J. In addition, it might be necessary to take weather conditions into account. However, all accessions showed significant differences (p<0.001) regarding the plant height throughout the years except for *CCPs- I* (p=0.360) (Table 14).

TABLE 14. Comparison of plant height (cm) difference over year in each accession

Year^1	B	A	G	D	E	H	I	J	L	C	K
1	*N/A*^{2}	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*	90.4 d	62.7
2					95.9 c	84.0 c	82.5	79.5	84.4	82.0 c	66.0
3				95.7 d	88.9 b	76.3 a	81.7	84.7		79.3 b	
4			91.6 d	87.4 c	86.8 ab	78.0 a	83.4			72.4 a	
5	94.7 c^3	92.5 c	80.9 bc	82.8 a	85.1 a	81.6 b				78.4 b	
6	86.2 b	85.8 b	79.1 b	80.7 a	84.5 a						
7	81.7 a	82.9 a	76.1 a	85.8 bc							
8	85.6 b	81.0 a	81.6 c								
9	86.1 b	83.0 a									
F-^{value}	30.46	25.99	45.82	41.49	25.00	23.19	1.02	21.66	-	97.30	31.15
P-^{value}	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.360	<0.001	-	<0.001	<0.001
LSD	2.271	2.345	2.286	2.284	2.465	1.909	-	-	-	1.756	-

[1] The number of repetition of CCPs cyclings

[2] N/A: Previous data not found

[3] Different letters within each cultivar (not between accessions) indicate a significant difference (P < 0.05) according to the LSD test.

† Grey color indicates the growing season 2015/2016

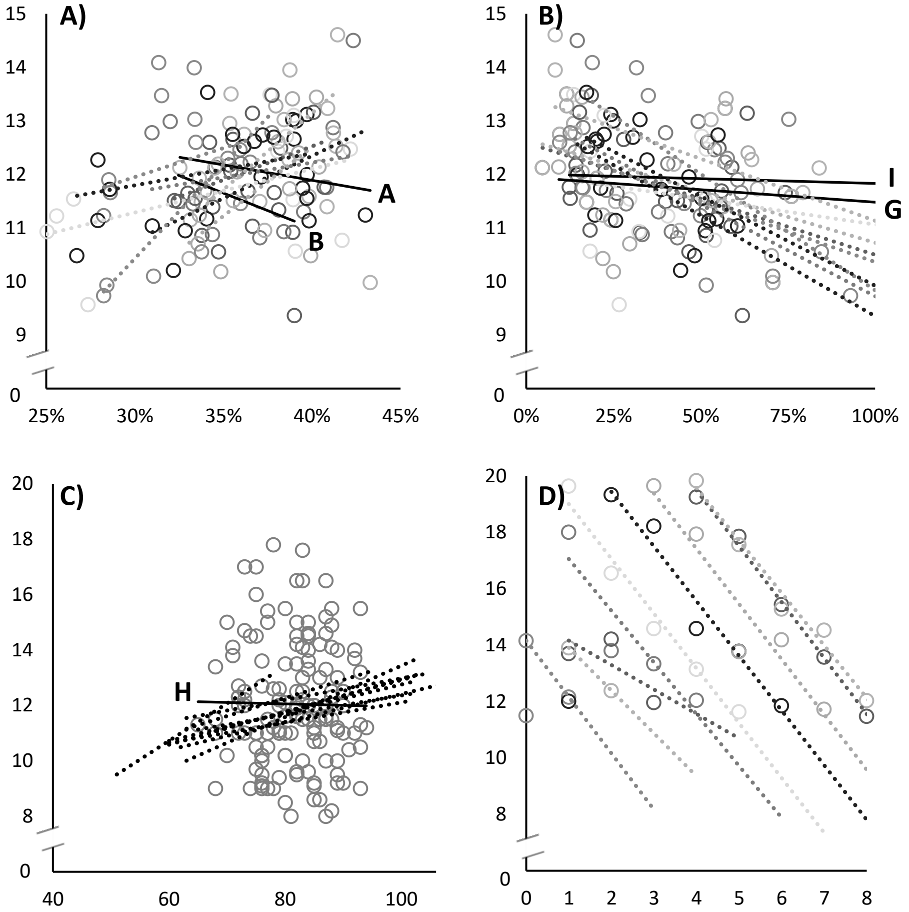


FIGURE 22. The linear regression model of flag leaf length in each CCPs cultivars and varieties. A) Ground cover rate and flag leaf length B) Weed density and flag leaf length C) Plant height and flag leaf length D) the number of CCPs cyclings and flag leaf length (Linear forecast was applied to the regression model of CCPs cyclings as each CCPs cultivars have different cycling years).

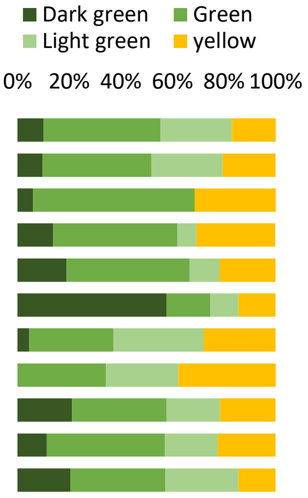
The flag leaf also showed a significant difference ($p<0.05$) in both the length and width of the flag leaf. The flag leaf area calculated according to the formula proposed by Stickler et al., (1961) did not show a significant difference ($p>0.05$) (Table 13). In case of the flag leaf length, except for the CCP- A and CCP- B (Figure 22 A), the leaf length of all accessions increased according to the increase of the ground cover rate. On the contrary, the flag leaf length decreased when the weed density increased except CCP- I and CCP- G (Figure 22 B). For the relationship between the flag leaf length and plant height, it showed that the higher the plant height is, the larger is the flag leaf length is except for CCP- H which shows almost constant length regardless of plant height (Figure 22 C). However, there was no regression between tillering and flag leaf in CCPs (data was not shown). Therefore, the overall flag leaf length (yi) of CCPs can be estimated by;

$y_i = 1.96 + 5.39\, x_{1i} - 2.10\, x_{2i} + 0.07\, x_{3i} + \varepsilon_i$, for i = 1,…9 with $\varepsilon_i \sim N(0,\sigma)$, independent from each other (where y_i=flag leaf length, x_1=ground cover rate, x_2=weed density, x_3=plant height)

In case of the distance between the flag leaf and spike, *CCP- E* showed the longest distance between the flag leaf and spike while *Julius (K)* showed the shortest distance (Table 13). It was generally suggested that a short distance between the flag leaf and spike might result in high susceptibility for spike diseases in wheat due to higher chances for spores to penetrate into the spike from the flag leaf. However, in this research, no significant relationship ($p>0.05$) was found between the distance and spike diseases (%) (data was not shown). Only *Naturastar (C)* showed higher spike disease symptoms among accessions (Table 13). In addition, the greenness of the flag leaf was calculated by using the image analysis. 50 flag leaves were randomly collected from each plot. The image analysis of each flag leaf was conducted using a web-based freeware program. On the basis of the color code, different color was calculated from the picture and similar color range was combined. Therefore, color category was divided into 4 groups; dark green, green, light green and yellow. There was a significant difference ($p=0.008$) in the greenness of the flag leaf among accessions (Table 15). *CCPs- H* showed the highest degree of greenness while *CCP- J* showed the lowest degree of greenness.

TABLE 15. The percentage of greenness of flag leaf extracted by image analysis

CCPs year		Color extraction (%) of flag leaf						
		Greenness						Yellow
		Dark	Green	Light	Total green		CV (%)	
9	B	10.2	45.3	27.6	83.0	ab[1]	17.2	17.0
9	A	9.8	42.1	27.5	79.4	ab	23.4	20.6
8	G	6.1	62.6	-	68.6	ab	21.8	31.4
7	D	13.8	48.2	7.3	69.4	ab	6.9	30.6
6	E	19.0	47.7	11.6	78.3	ab	7.8	21.7
5	H	57.8	17.0	10.8	85.6	b	14.1	14.4
4	I	4.6	32.6	35.0	72.2	ab	7.1	27.8
3	J	-	34.3	28.1	62.3	a	31.9	37.7
2	L	21.2	36.6	20.7	78.5	ab	23.8	21.5
-	C	11.4	45.8	20.4	77.6	ab	10.5	22.4
-	K	20.6	36.7	28.4	85.7	ab	15.1	14.3
F-value					2.63			
P-value					0.008			
LSD					Bonferroni test[2]			



[1] Different letters on characteristic indicate a significant difference (P<0.05) according to the LSD test

[2] Multiple comparisons under non-parametric data

TABLE 16. Comparison of flag leaf length (cm) difference over year in each accession

Year[1]	B	A	G	D	E	H	I	J	L	C	K
1	*N/A*[2]	*N/A*	*N/A*	*N/A*	*N/A*	N/A	*N/A*	*N/A*	*N/A*	20.7 d	11.5
2					19.7 d	18.0 c	*N/A*	13.9	12.0	18.0 c	11.5
3				19.3 d	16.6 c	*N/A*	14.2	12.4		*N/A*	
4			19.7 d	18.2 c	*N/A*	13.3 b	12.0			13.9 b	
5	19.8 d[3]	19.3 d	17.9 c	*N/A*	13.2 b	12.0 a				12.2 a	
6	17.9 c	17.6 c	*N/A*	13.8 b	11.6 a						
7	*N/A*	*N/A*	14.2 b	11.9 a							
8	14.5 b	13.6 b	11.7 a								
9	12.0 a	11.5 a									
F-^{value}	214.85	216.52	222.81	194.21	223.71	196.86	43.89	20.79	-	227.20	1.35
P-^{value}	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	-	<0.001	0.246
LSD	0.6546	0.670	0.6657	0.7052	0.6565	0.6103	-	-	-	0.7073	-

[1] The number of CCPs cyclings

[2] N/A: Previous data not found

[3] Different letters within each cultivar (not between accessions) indicate a significant difference ($P < 0.05$) according to the LSD test.

† Grey color indicates the growing season 2015/2016

Previous data of growing seasons were also analyzed in order to show the trend of flag leaf length over the years. Similar to the plant height, the flag leaf length also decreased over CCPs cyclings (Figure 22 D). All accessions showed significant differences ($p<0.001$) in the flag leaf length over years except for *Julius (K)* (Table 16). However, when the genotype performance is evaluated, each year has different environmental and climate conditions. Therefore, a simple comparison of the mean amongst years might have limitations to fully explain the genotype differences. As an alternative way, the relative mean of several late vegetative traits such as plant height, flag leaf length and the distance (flag leaf to spike) in each year were calculated and compared to each other. All CCPs and *Naturastar (C)* did not show any significant differences of relative mean ($p>0.05$) in each growing season with respect to flag leaf length (Figure 23 C) On the other hand, the plant height and the distance between the flag leaf and spike showed significant differences ($p<0.05$) in several growing seasons (Figure 23 A). In growing season 2015/2016, there was no significant differences ($p>0.05$) of all relative means in late vegetative traits (Figure 23 A,B,C)

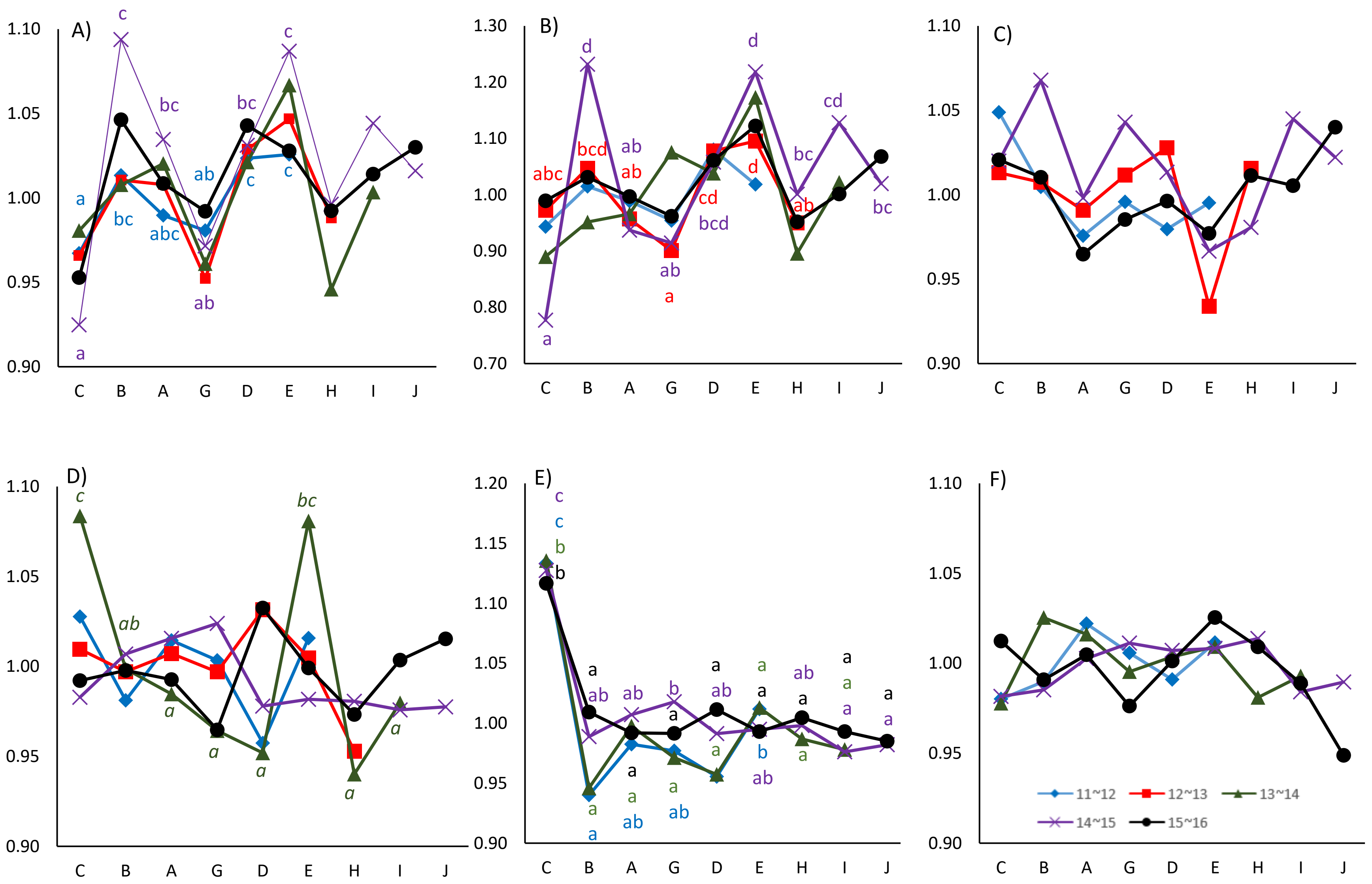


FIGURE 23. The relative mean for A) Plant height B) Distance between flag leaf and spike C) Flag leaf length D) Spike length E) Number of spikelet F) Spike fertility (%) from the growing season 2011/2012 to 2015/2016. †Different letters between cultivars indicate a significant difference (P<0.05) according to the LSD test.

TABLE 17. The summary tables for yield components in growing season 2015/2016

		Yield components																
		Number of fertile tillers		Spike length			Number of spikelet per spike						Spikelet density per spike			Spike fertility		
							Total			Fertile								
CCPs Year		Mean (#)	CV (%)	Mean (cm)		CV (%)	Mean (#)		CV (%)	Mean (#)		CV (%)	Mean (%)		CV (%)	Mean (%)		CV (%)
9	B	315	11.3	7.1	cd[1]	8.8	17.9	de	8.8	13.4	c	12.2	60.2	de	7.1	75.0	bcd	7.8
9	A	295	11.3	7.0	bc	12.0	17.6	bcde	9.2	13.4	c	15.0	59.9	d	6.4	76.1	bcd	8.7
8	G	330	20.8	6.8	a	11.1	17.6	bcd	9.2	13.0	abc	14.1	61.0	ef	5.4	73.9	ab	8.6
7	D	321	12.0	7.3	ef	9.7	17.9	e	9.5	13.6	c	17.1	59.0	bc	5.9	75.8	bcd	11.8
6	E	295	8.8	7.1	cd	8.9	17.6	bcde	8.5	13.7	c	12.6	59.6	cd	5.7	77.6	de	7.7
4	H	308	7.8	6.9	ab	11.8	17.8	cde	10.2	13.6	c	14.5	61.1	f	6.8	76.4	bcd	8.3
3	I	312	12.2	7.1	cd	9.7	17.6	bcde	8.2	13.2	abc	12.4	59.4	bcd	6.9	74.9	bc	7.6
2	J	299	6.0	7.2	de	10.0	17.4	bc	9.2	12.5	a	16.5	58.6	b	6.7	71.8	a	13.7
1	L	289	12.8	7.2	cde	9.8	17.4	b	8.9	13.3	bc	13.0	58.6	b	6.7	76.2	bcd	8.4
-	C	293	17.4	7.0	bc	12.7	19.8	f	8.5	15.2	d	13.8	64.4	g	5.6	76.6	cd	8.0
-	K	261	11.3	7.3	f	12.3	15.7	a	10.3	12.5	ab	14.7	53.5	a	7.5	79.8	e	8.3
	$F^{\text{-value}}$	1.76		7.77			51.09			22.07			56.54			13.25		
	$P^{\text{-value}}$	*0.138*		*<0.001*			*<0.001*			*<0.001*			*<0.001*			*<0.001*		
	LSD	-		0.1600			0.3000			Bonferroni test[2]			0.0088			Bonferroni test		

[1] Different letters within on characteristic indicate a significant difference (P<0.05) according to the LSD test

[2] Multiple comparisons under non-parametric data

3.3. Reproductive traits

The result of the reproductive traits was summarized in Table 17.

3.3.1. Flowering date

The date of flowering of each accession was investigated since the inflorescence emergence. As CCPs were highly heterogeneous, it was difficult to decide the exact flowering stage. However, all accessions were observed consistently as a whole, not as single plants for the most accurate flowering date. In growing season 2015/2016, all CCPs had the same day with respect to the Inflorescence emergence (heading) which was around mid-May. On the other hand, two varieties were a week late compared with CCPs (Figure 24). Compared to the growing season 2014/2015, the 50% and 100% flowering was delayed for 2~4 days. What is more, in comparison with growing season 2011/2012, the 50% flowering was delayed for about two weeks in CCPs and *Naturastar (C)*. It can be inferred that the delayed flowering was affected by environment variability over times. However, genetic evolution can be also the factor to delay flowering, in that even though *Naturastar (C)* is homozygous, it has been exposed to CCPs cycling for a certain period of time and heterozygosity might be increased. At any rate, the time of 50% flowering in 2011/2012 is the time of inflorescence development in 2015/2016. It is obvious that the reproductive stage has been delayed due to environmental variability (Appendix 6).

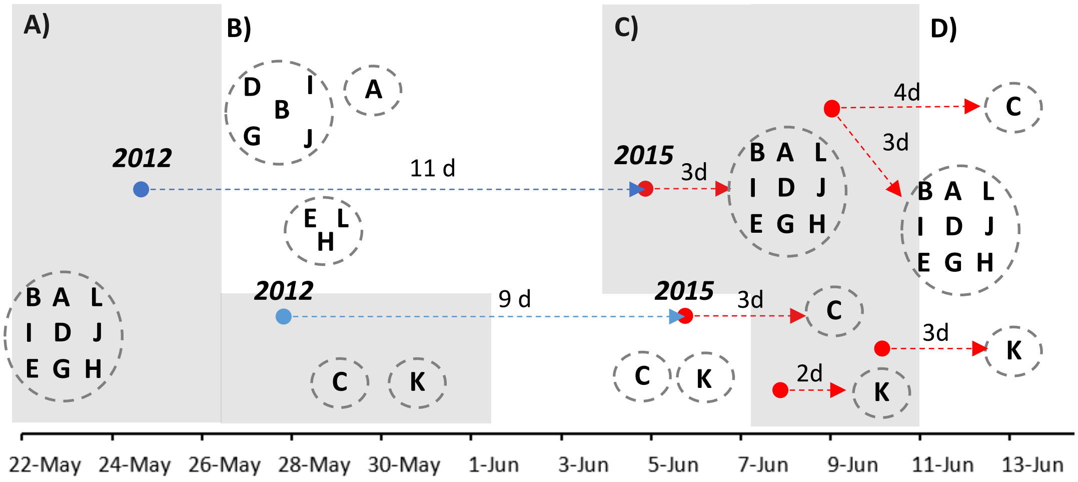


FIGURE 24. Flowering data of CCPs cultivars and varieties in the growing season 2015/2016. A) The stage of inflorescence emergence B) The stage of flowering initiation C) The stage of 50% flowering D) The stage of 100% flowering. Arrow indicates delay of flowering from previous year

3.3.2. Fertile tillers

The number of fertile tillers was investigated in the development of fruit stage (BBCH-scale: 71 to 77). Early and late tillering was already investigated in the tillering stage (BBCH-scale: 20 to 29) and the tillering number showed a significant difference ($p<0.001$) during both stages. However, there was no significant difference ($p=0.138$) in fertile tillering among accessions.

3.3.3. Spike characteristics

Several different traits related to spike characteristics were investigated. All significant differences ($p<0.001$) were found and *Julius (K)* showed the longest spike length (Table 17). However, *Julius (K)* showed poor performance in this season and the longer spike might result from the border effect which allows plants to have more space and nutrients. On the contrary, *Julius (K)* showed the lowest number of spikelets per spike. In case of spikelet density, it was expected that the higher the spike density, the less air is circulated throughout spike and it might bring out high risks for ear fungi (Figure 25 A). In this research, a positive correlation ($p=0.014$) between spikelet density and spike diseases (%) was also found (Figure 25 B). *Naturastar (C)*, showed the highest spikelet density and so, also the highest spike disease (%) while *Julius (K)* showed the opposite result (Table 13, Table 17) . For the spike fertility (%), *CCP- J* showed the lowest spike fertility and the highest fertility was shown in *Julius (K)*.

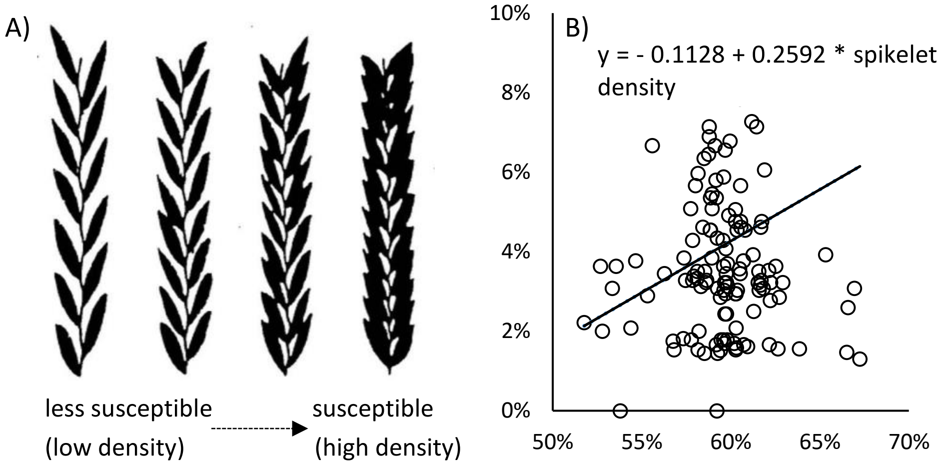


FIGURE 25. Relationship spike disease and plant morphology. A) Accessions with compact spikes are susceptible for fungi B) Regression model between spikelet density (x-axis) and spike diseases (%) (y-axis) in a population of growing season 2015/2016.

Actually, the number of spikelets affected several traits such as the spike fertility and density. Therefore, as a multiple regression model; $y_i = \beta_0 + \beta_1 x_{1i} - \beta_2 x_{2i} + \varepsilon_i$, the overall total number of spikelet (y_i) of CCPs can be estimated by;

$y_i = 8.14 + 1.37\, x_{1i} - 0.01\, x_{2i} + \varepsilon_i$, for i = 1,…9 with $\varepsilon_i \sim N(0,\sigma)$, independent from each other (where y_i= total number of spikelet, x_1= spike length, x_2= distance b/t flag leaf and spike)

According to the multiple regression model, the number of spikelets can be decreased when the distance between the flag leaf and spike is increased.

Also, previous data of growing seasons from 2012 to 2015 were also analyzed to compare the difference of spike fertility (%). All accessions showed a significant difference ($p<0.001$) in spike fertility over years (Table 18). In order to compare genotype performances over years, a relative mean of reproductive traits (spike length, number of spikelet and spike fertility) was also calculated. All CCPs and *Naturastar (C)* did not show any significant differences of relative mean ($p>0.05$) in each growing season with respect to spike fertility (Figure 23 F). Also, it showed a similar pattern of relative mean over the years with respect to the number of spikelets (Figure 23 E). On the other hand, the spike length did not show a similar pattern of the relative mean over the years (Figure 23 D).

TABLE 18. Comparison of spike fertility (%) difference over year in each accession

Year[1]	B	A	G	D	E	H	I	J	L	C	K
1	*N/A*[2]	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*	78.3 b	77.8
2					80.8 b	*N/A*	80.1 b	75.8	76.2	*N/A*	79.8
3				79.1 cd	*N/A*	78.9 b	75.3 a	71.8		78.4 b	
4			80.3 c	*N/A*	81.7 b	77.6 ab	74.9 a			75.1 a	
5	79.0 b[3]	81.6 b	*N/A*	80.6 d	77.2 a	76.4 a				76.6 ab	
6	*N/A*	*N/A*	79.9 c	77.1 bc	77.6 a						
7	81.3 c	81.1 b	77.4 b	75.8 ab							
8	75.4 a	76.7 a	73.9 a								
9	75.0 a	76.1 a									
F-$^{\text{value}}$	64.73	67.73	61.92	45.03	95.94	69.02	17.19	12.91	-	65.28	5.64
p-$^{\text{value}}$	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	-	<0.001	0.018
LSD	0.019	0.019	0.020	0.022	0.017	0.019	0.019	-	-	0.021	-

[1] The number of repetition of CCPs cyclings

[2] N/A: Previous data not found

[3] Different letters within each cultivar (not between accessions) indicate a significant difference ($P<0.05$) according to the LSD test.

†† Grey color indicates the growing season 2015/2016

3.4. Yield

The yield (ton·ha^{-1}) and TWK (g) were measured per each sub-plot. There were significant differences ($p<0.001$) in yield (ton·ha^{-1}) and TKW (g) among accessions (Table 19). When all accessions were harvested, CCPs showed a higher yield (2.42 ~ 2.77 ton·ha^{-1}) than varieties (1.74 ~ 2.09 ton·ha^{-1}). When adjusted yield (ton·ha^{-1}) was applied, CCPs also showed higher yield (ton·ha^{-1}) than varieties. For TKW (g), *Naturastar (C)* showed the lowest thousand kernel weight (28.63 g). Although *Julius (K)* showed the lowest yield (ton·ha^{-1}), it also showed relatively the highest thousand kernel weight (36.96 g). In this experiment, *Julius (K)* exhibited a low plant density which results from plot damage or low vigor. Therefore, *Julius (K)* might have more border effect which indicates that each plant less competes with adjacent plants. It might have resulted in the highest TKW (g) of *Julius (K)*.

Undoubtedly, a positive correlation was shown ($p<0.001$) between TWK (g) and the harvested yield (ton·ha^{-1}) among accessions (Figure 26 A). On the other hand, there was a negative correlation ($p=0.032$) between the greenness of the flag leaf and TKW (g) among accessions (Figure 26 B).

TABLE 19. Summary of yield and TWK in growing season 2015/2016

CCPs Year	Accession	Yield (ton·ha^{-1})				TKW (g)	
		Harvested	CV (%)	Adjusted[1]	CV (%)	Mean	CV (%)
9	B	2.74 c[2]	16.6	3.02 cd	21.2	35.96 cd	0.06
9	A	2.50 c	25.0	2.59 bc	25.4	34.70 bcd	0.10
8	G	2.77 c	18.9	3.18 d	27.2	36.15 cd	0.10
7	D	2.66 c	33.1	2.82 cd	29.3	36.62 cd	0.08
6	E	2.42 bc	32.1	2.59 bc	30.2	35.45 bcd	0.08
4	H	2.57 c	10.4	2.77 cd	7.2	32.88 b	0.12
3	I	2.69 c	11.5	2.90 cd	15.0	34.11 bc	0.07
2	J	2.52 c	26.6	2.87 cd	37.5	37.06 d	0.08
1	L	2.57 c	34.5	2.88 cd	34.8	35.87 cd	0.13
-	C	2.09 ab	26.6	2.28 ab	23.2	28.63 a	0.13
-	K	1.74 a	48.9	2.06 a	55.0	36.96 cd	0.11
	F-value	4.49		4.48		6.74	
	p-value	<0.001		<0.001		<0.001	
	LSD	0.409		0.43		2.58	

[1] adjusted mean = harvested yield + harvested yield x plot damage (%)

[2] Different letters within on characteristic indicate a significant difference ($P<0.05$) according to the LSD test

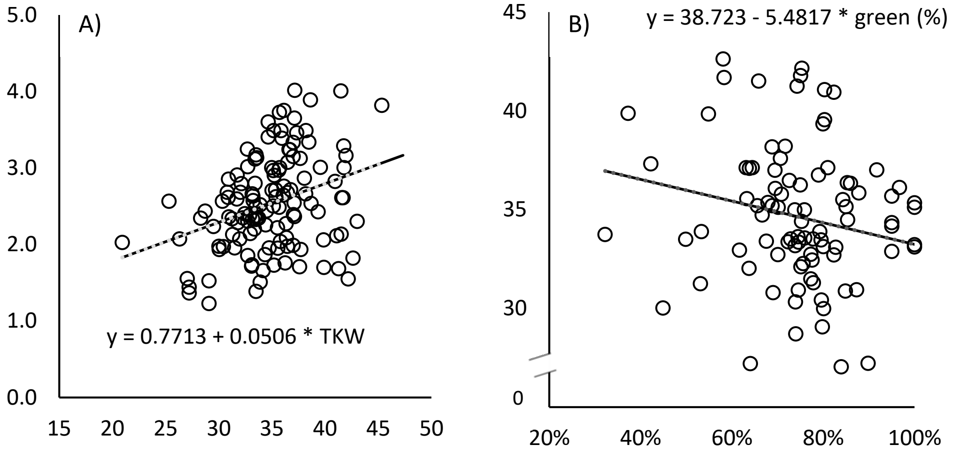


FIGURE 26. Linear regression model between A) TKW (g) and yield (ton·ha^{-1}) B) Greenness of flag leaf (%) and TKW (g)

In order to elucidate which traits actually affect the final yield in CCPs, as a multiple regression ($p<0.001$) model; $y_i = \beta_0 + \beta_1 x_{1i} - \beta_2 x_{2i} + \beta_3 x_{3i} + \cdots \beta_8 x_{8i} + \varepsilon_i$, yield (ton·ha^{-1}) of CCPs was estimated by

$y_i = -1.238 - 0.002\, x_{1i} + 1.278\, x_{2i} + 0.094\, x_{3i} + 0.163\, x_{4i} - 0.006\, x_{5i} - 0.794\, x_{6i} + 0.026\, x_{7i} + 0.554\, x_{8i} + \varepsilon_i$, for i = 1,…9 with $\varepsilon_i \sim N(0,\sigma)$, independent from each other

(where y_i=yield, x_1=early tillering, x_2=early ground cover rate, x_3=plant height, x_4=flag leaf length, x_5=distance between flag leaf and spike, x_6= spike length, x_7=number of spikelet, x_8=spike fertility)

In this regression model, early tillering, distance (flag leaf to spike) and spike length showed a negative correlation while the ground cover rate, plant height, flag leaf length, number of spikelet and spike fertility exhibited positive influences on the yield.

Also, previous data of growing seasons from 2012 to 2015 were also analyzed to compare the differences of yield and TKW. In CCPs, there were no significant differences ($p>0.05$) of CCPs over the years in *CCP-B*, *CCP-A*, *CCP-H* and *CCP-J* (Table 20), while in TKW, no significant difference ($p=0.411$) was only shown in *CCP-J* (Table 21). In case of varieties, only *Naturastar (C)* showed significant difference ($p<0.05$) of yield and TKW over the years.

TABLE 20. Comparison of yield (ton·ha^{-1}) difference over year in each accession

Year[1]	B	A	G	D	E	H	I	J	L	C	K
1	*N/A*[2]	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*	4.22 b	1.50
2					4.16 c	2.75	3.40 b	1.39	2.88	1.88 a	2.06
3				3.89 c	2.04 a	3.15	1.65 a	2.87		2.91 a	
4			4.10 c[3]	1.98 ab	3.45 bc	1.84	2.90 b			1.55 a	
5	3.88	3.78	1.71 a	3.23 bc	1.50 a	2.77				2.28 a	
6	2.07	2.29	3.26 bc	1.42 a	2.59 ab						
7	2.65	3.18	1.79 ab	2.82 abc							
8	1.72	1.89	3.18 bc								
9	3.02	2.59									
F-$^{\text{value}}$	3.06	3.67	6.75	5.43	7.33	2.01	11.54	1.1	-	8.16	0.15
p-$^{\text{value}}$	0.083	0.055	0.011	0.026	0.012	0.215	0.022	0.404	-	0.009	0.739
LSD	-	-	1.165	1.159	1.12	-	0.714	-	-	1.118	-

TABLE 21. Comparison of TKW (g) difference over year in each accession

Year[1]	B	A	G	D	E	H	I	J	L	C	K
1	*N/A*[2]	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*	34.3 b	39.8
2					39.5 b	38.0 bc	43.2 b	33.8	35.9	35.8 b	37.0
3				39.4 ab	41.0 bc	39.8 c	36.7 a	37.1		37.6 b	
4			38.1 ab[3]	41.7 b	42.1 c	34.5 ab	34.1 a			33.8 ab	
5	38.0 ab[3]	39.0 b	39.6 b	42.8 b	34.5 a	32.9 a				28.6 a	
6	40.8 b	41.3 b	41.0 b	34.2 a	35.5 a						
7	41.3 b	41.4 b	34.7 a	36.6 a							
8	35.4 a	36.4 ab	36.1 a								
9	36.0 a	34.7 a									
F-$^{\text{value}}$	4.65	6.54	4.16	5.01	19.61	8.99	43.75	1.06	-	5.75	3.19
p-$^{\text{value}}$	*0.031*	*0.012*	*0.041*	*0.032*	*<.001*	*0.012*	*0.002*	*0.411*	-	*0.023*	*0.325*
LSD	3.635	3.591	3.374	4.071	2.071	3.503	2.746	-	-	4.464	-

[1] The number of repetition of CCPs cycling

[2] N/A: Previous data not found

[3] Different letters within each cultivar (not between accessions) indicate a significant difference (P<0.05) according to the LSD test.

† Grey color indicates the growing season 2015/2016.

Furthermore, the relative means of yield and TKW were calculated in order to compare the yield performance of each year relatively. For yield, all CCPs and *Naturastar (C)* showed significant differences of the relative mean ($p<0.05$) only in the growing season 2012/2013 (Figure 27 A) while TKW showed significant differences ($p<0.05$) of relative mean in all growing seasons (Figure 27 B).

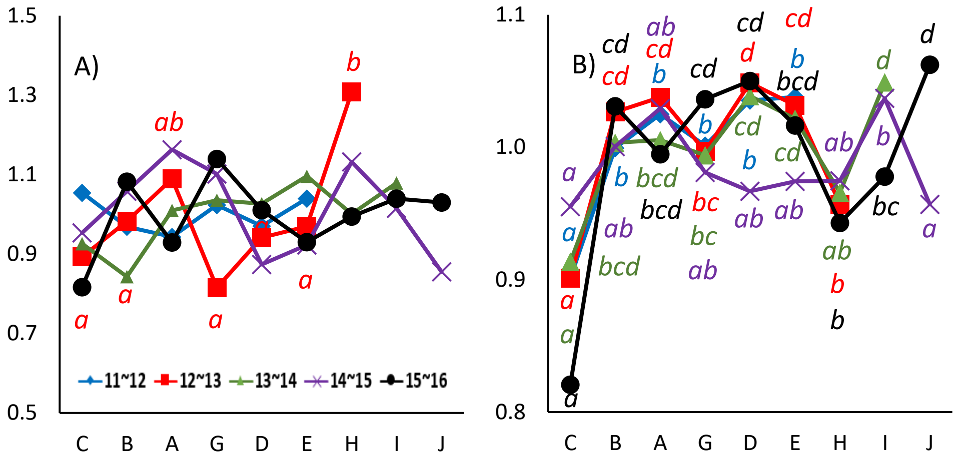


FIGURE 27. The relative mean for A) Yield B) TKW from the growing season 2011/2012 to 2015/2016. † Different letters between cultivars indicate a significant difference (P<0.05) according to the LSD test.

3.5. Heritability

The heritability was shown in Table 22. Heritability (h^2) is generally defined as the ratio of the genetic variance to the total phenotypic variance (genetic variance + non-genetic variance) in the plant material. A high heritability means that the variation which is observed in the present materials is caused by variation in genotypes. In other words, it means that the phenotype of an individual plant is a good predictor of the genotype in the current materials (Visscher *et al.,* 2008). Furthermore, heritability can be also defined as the experimental result under repeatable conditions where independent results are obtained with the same experimental equipment or operator within short interval of time. High heritability indicates that repeated measures of the same individual plant have less variation (Horie *et al.,* 2011; Boake, 1989). In terms of $R = h^2 \times S$, when h^2 increases, it results in a higher selection response (R) which means genetic advance. In the early growing stage, early vegetative traits such as seedling emergence, ground cover rate and tillering showed low heritability. It indicates that early vegetative traits are more affected by environmental conditions. In addition, the leaf length and width showed a relatively low heritability. Yield and TKW also showed relatively low heritability. On the other hand, yield components except TKW and late vegetable traits such as the plant height and the distance (leaf and spike) showed relatively high heritability. It can be inferred that, in the early growing stage, non-genetic variance is larger than genetic variance and vice versa in the late growing stage. Therefore, it might be important to maintain favorable farming conditions in the early growing stage to reduce non-genetic variances such as environmental errors. The yield and TKW could be more affected by

TABLE 22. Heritability of each trait of accessions in growing season 2015/2016

	CCPs [9]					Variety [2]				
	MS_G (σG^2)		MS_E	Heritability	CV	MS_G (σG^2)		MS_E	Heritability	CV
Traits	Normal	Expected[1]	(σE^2)	(%)	(%)	Normal	Expected	(σE^2)	(%)	(%)
Seedling emergence	63.72	12.96	24.84	34.3	7.2	2281.50	755.15	16.04	97.9	6.4
Ground cover rate										
Early	0.0014	0.0000	0.0014	0.8	16.6	0.0177	0.0054	0.0015	78.7	20.0
Late	0.0017	0.0004	0.0005	45.0	10.3	0.0073	0.0015	0.0027	35.9	17.6
Tillering										
Early	188.80	*N/A*[2]	222.70	*N/A*	13.6	1980.20	590.50	208.70	73.9	18.0
Late	192.20	28.40	107.00	21.0	14.6	1024.00	312.78	85.66	78.5	15.7
Fertile	93.17	13.46	52.78	20.3	11.5	306.25	80.43	64.97	55.3	14.4
Flag leaf										
Length	10.38	1.99	4.41	31.1	18.1	22.00	5.73	4.80	54.5	19.6
Width	0.0402	0.0076	0.0173	30.6	13.3	0.1819	0.0550	0.0170	76.4	13.3
Plant height	395.07	35.23	119.95	77.3	9.0	6555.12	20.09	2178.34	99.1	7.9
Distance (leaf to spike)	111.06	31.00	18.07	63.2	26.8	958.78	316.95	7.94	97.6	19.9
Yield components										
Spike length	3.74	1.08	0.51	68.1	10.2	12.01	3.83	0.51	88.2	12.5
No. of spikelet	11.51	3.00	2.51	54.4	9.1	848.72	282.18	2.17	99.2	9.4
Spikelet density	0.0092	0.0026	0.0015	63.9	9.5	0.7520	0.2503	0.0012	99.5	9.4
Spike fertility	0.0279	0.0077	0.0048	61.6	9.2	0.0969	0.0311	0.0036	89.7	8.2
TKW	20.74	3.77	9.42	28.6	9.6	367.80	117.59	15.04	88.7	17.6
Yield	0.42	0.06	0.24	20.0	26.6	0.11	*N/A*	0.13	*N/A*	23.2

[1] Expected mean square: (MS_G - MS_E) / r, where r=3

[2] N/A: Heritability can not be calculated because MS_E is bigger than MS_G

non-genetic variance due to low heritability. Therefore, harvest and post-harvest management might be also important to reduce environmental errors. For varieties, it showed higher heritability than CCPs in several traits. However, only 2 pure line varieties were compared with 9 CCPs. It was unbalanced to compare G x E interaction between CCPs and varieties. Therefore, higher heritability in varieties might not indicate higher selection response (R). In order to elucidate precise G x E interaction, more varieties are necessary to be added for further researches.

3.6. Shannon-Weaver Diversity Index and Awn emergence

The relative diversity index (H') of individual traits is shown in Table 23. The Shannon-Weaver Diversity Index assumes that individuals are randomly sampled from an independent large population, and all the species are represented in the sample (Shannon and Weaver, 1949) and the index explains species richness and evenness that contribute to the diversity (Ortiz-Burgos, 2016). For growing season 2015/2016, there was a significant difference among CCPs and two varieties in the plant height, distance (the flag leaf and spike) and the total spikelet. Peculiarly, *Julius (K)* showed relatively the highest values in those traits. Except *Julius (K)*, other accessions showed similar relative values of the diversity index. In this growing season, however, *Julius (K)* showed extremely low performance in the field compared to other accessions. It was considered as impractical to calculate diversity index including *Julius (K)*. Therefore, *Julius (K)*

TABLE 23. Relative mean on Shannon-Weaver Diversity Index and awn (%) for growing season 2015/2016

	Relative mean on Shannon-Weaver Diversity												Awn (%)	
	Plant height		Distance[1]		Leaf length	Leaf width	Spike length	No. of spikelet		Spikelet density	Spike fertility		Early	Late
B	0.88	a[2]	0.98	ab	0.99	1.00	0.99	1.00	b	1.02	0.98		5.4 f	8.9 f
A	0.97	abc	1.03	ab	1.03	1.02	1.00	0.99	b	1.01	0.99		2.3 bc	4.4 bc
G	1.01	abc	1.02	ab	0.99	0.98	1.05	1.00	b	1.01	1.01		3.4 cde	2.6 b
D	0.93	abc	0.97	ab	1.00	1.00	0.96	0.97	b	0.97	1.01		3.8 de	7.4 def
E	0.94	abc	0.94	a	1.01	1.00	0.99	1.00	b	0.99	0.94		3.5 cde	6.2 cde
H	1.03	bc	1.02	ab	0.99	1.00	1.02	0.99	b	1.03	0.97		1.9 b	4.3 bc
I	0.96	abc	0.99	ab	1.00	1.00	0.97	0.99	b	1.00	0.99		4.8 ef	8.0 ef
J	0.93	ab	0.97	ab	0.99	1.00	0.97	1.02	b	0.98	1.09		2.5 bcd	5.6 cd
L	0.92	ab	0.97	ab	0.99	1.00	0.99	1.03	b	0.97	0.97		1.6 b	2.4 b
C	1.07	c	0.99	ab	0.99	0.99	1.01	0.84	a	1.09	0.95		0.1 a	0.1 a
K	~~*1.35*~~	*d*	~~*1.12*~~	*c*	~~*1.03*~~	~~*1.01*~~	~~*1.05*~~	~~*1.16*~~	*c*	~~*0.94*~~	~~*1.11*~~		0.0 a	0.0 a
F-^{value}	7.110		4.130		1.260	0.230	0.890	6.71		1.140	0.530		11.09	16.55
P-^{value}	<.001		0.003		*N.S*[3]	*N.S*	*N.S*	<.001		*N.S*	*N.S*		*<.001*	*<.001*

[1] Distance between flag leaf and spike

[2] Different letters within on characteristic indicate a significant difference (P<0.05) according to the LSD test

[3] not significant

TABLE 24. Genotype x Year interaction based on different growing periods

				Sig.	Plant height	Flag leaf		Distance (flag leaf to spike)	Yield components				Yield
						length	width		Spike length	No. of spikelet	Spike fertility	TKW	
2012 ~ 2016	CCPs [9]	*G x Y Interaction*		$F^{\text{-value}}$	2.66	2.07	2.37	2.63	4.03	2.97	2.59	0.51	0.39
				$p_{\text{-value}}$	*< 0.001*	*0.007*	*0.002*	*< 0.001*	*< 0.001*	*< 0.001*	*< 0.001*	*0.955*	*0.991*
		Main effect	G	$F^{\text{-value}}$	40.04	72.7	116.64	21.36	18.24	5.56	14.48	2.94	0.24
				$p_{\text{-value}}$	*< 0.001*	*< 0.001*	*< 0.001*	*< 0.001*	*< 0.001*	*< 0.001*	*< 0.001*	*0.009*	*0.982*
			Y	$F^{\text{-value}}$	198.37	1404.54	1630.11	172.34	197.67	61.51	103.36	40.37	24.09
				$p_{\text{-value}}$	*< 0.001*	*< 0.001*	*< 0.001*	*< 0.001*	*< 0.001*	*< 0.001*	*< 0.001*	*< 0.001*	*< 0.001*
	Variety [2]	*G x Y Interaction*		$F^{\text{-value}}$	2.07	12.51	0.36	35.19	5.03	0.13	0.27	0.4	0.02
				$p_{\text{-value}}$	*0.151*	*< 0.001*	*0.549*	*< 0.001*	*0.025*	*0.718*	*0.601*	*0.547*	*0.898*
		Main effect	G	$F^{\text{-value}}$	737.18	412.26	2031.61	370.22	0.05	645.21	48.89	8.33	3.05
				$p_{\text{-value}}$	*< 0.001*	*< 0.001*	*< 0.001*	*< 0.001*	*0.826*	*< 0.001*	*< 0.001*	*0.02*	*0.115*
			Y	$F^{\text{-value}}$	221.35	388.19	7502.15	193.52	57.86	985.96	78.32	3.46	5.28
				$p_{\text{-value}}$	*< 0.001*	*< 0.001*	*< 0.001*	*< 0.001*	*< 0.001*	*< 0.001*	*< 0.001*	*0.064*	*0.018*
2012 ~ 2014	CCPs [8]	*G x Y Interaction*		$F^{\text{-value}}$	1.67	3.39	4.02	2.42	3.18	0.18	1.42	0.13	0.82
				$p_{\text{-value}}$	*0.09*	*0.007*	*0.003*	*0.01*	*< 0.001*	*0.949*	*0.224*	*0.998*	*0.606*
		Main effect	G	$F^{\text{-value}}$	30.8	2.93	11.9	8.65	6.74	8.88	1.43	2.59	0.57
				$p_{\text{-value}}$	*< 0.001*	*0.012*	*< 0.001*	*< 0.001*	*< 0.001*	*< 0.001*	*0.201*	*0.036*	*0.752*
			Y	$F^{\text{-value}}$	216.65	121.54	170.51	18.77	16.93	54.1	8.13	10.15	52.54
				$p_{\text{-value}}$	*< 0.001*	*< 0.001*	*< 0.001*	*< 0.001*	*< 0.001*	*< 0.001*	*0.004*	*< 0.001*	*< 0.001*

				Sig.	Plant height	Flag leaf		Distance (flag leaf to spike)	Yield components				Yield
						length	width		length	No. of spikelet	fertility	TKW	
2015 ~ 2016	CCPs [9]	*G x Y Interaction*		$F^{\text{-value}}$	4.33	2.14	1.44	3.67	5.95	2.09	3.37	0.7	0.07
				$p_{\text{-value}}$	*< 0.001*	*0.036*	*0.184*	*< 0.001*	*< 0.001*	*0.041*	*0.001*	*0.671*	*0.999*
		Main effect	G	$F^{\text{-value}}$	15.06	4.81	3.89	*12.28*	2.47	3.91	7.2	0.7	0.18
				$p_{\text{-value}}$	*< 0.001*	*< 0.001*	*< 0.001*	*< 0.001*	*0.011*	*< 0.001*	*< 0.001*	*0.687*	*0.991*
			Y	$F^{\text{-value}}$	57.88	321.64	219.34	307.8	334.29	126.18	28.36	0.22	12
				$p_{\text{-value}}$	*< 0.001*	*< 0.001*	*< 0.001*	*< 0.001*	*< 0.001*	*< 0.001*	*< 0.001*	*0.642*	*0.003*
	Variety [2]	*G x Y Interaction*		$F^{\text{-value}}$	0.42	18.03	9.75	34.23	3.32	0.09	0.67	3.18	0.01
				$p_{\text{-value}}$	*0.517*	*< 0.001*	*0.002*	*< 0.001*	*0.069*	*0.763*	*0.413*	*0.173*	*0.922*
		Main effect	G	$F^{\text{-value}}$	258.71	37.94	2.68	109.04	55.17	629.17	14.8	13.59	0.06
				$p_{\text{-value}}$	*< 0.001*	*< 0.001*	*0.102*	*< 0.001*	*< 0.001*	*< 0.001*	*< 0.001*	*0.035*	*0.813*
			Y	$F^{\text{-value}}$	106.56	9.79	12.34	325.9	97.32	20.99	4.41	4.53	0.64
				$p_{\text{-value}}$	*< 0.001*	*0.002*	*< 0.001*	*< 0.001*	*< 0.001*	*< 0.001*	*0.036*	*0.123*	*0.469*

was excluded in the calculation of the Shannon-Weaver Diversity Index (data was not shown). When *Julius (K)* was ruled out, all CCPs did not show significant difference of the Shannon-Weaver Diversity Index in the growing season 2015/2016. In case of awn emergence, varieties showed lower awn (%) than CCPs. Naturastar (C) showed small emergence of awn but any awn emergence was not found at all in Julius (K). On the contrary, the oldest CCP; *CCP- B* showed the highest awn emergence (Table 23).

3.7. Genotype x Year interaction

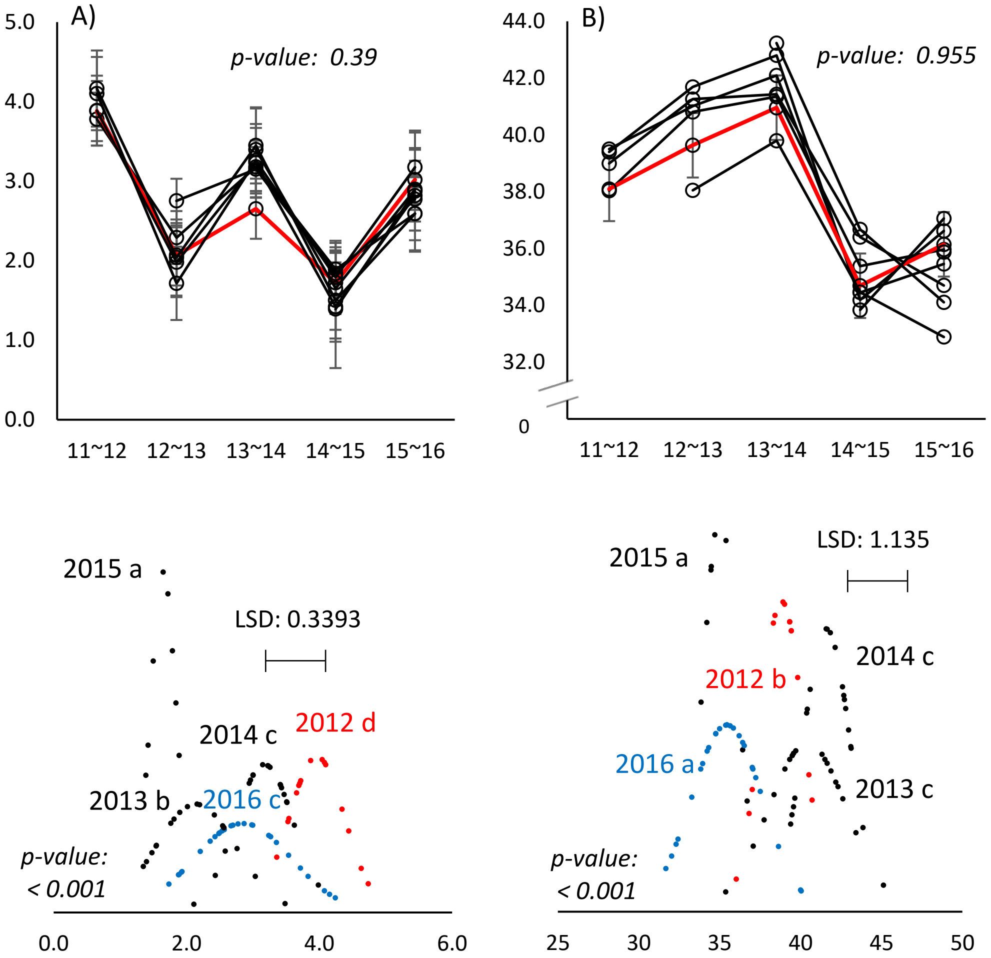


FIGURE 28. Genotype variance over year in CCPs (Top: genotype variance over year, Bottom: CCPs population variance over year) A) Yield B) TKW. † top: red line indicates the oldest CCPs (CCPs- B), bottom: red line indicates 2011/2012 growing season and blue line indicates 2015/2016 growing season.

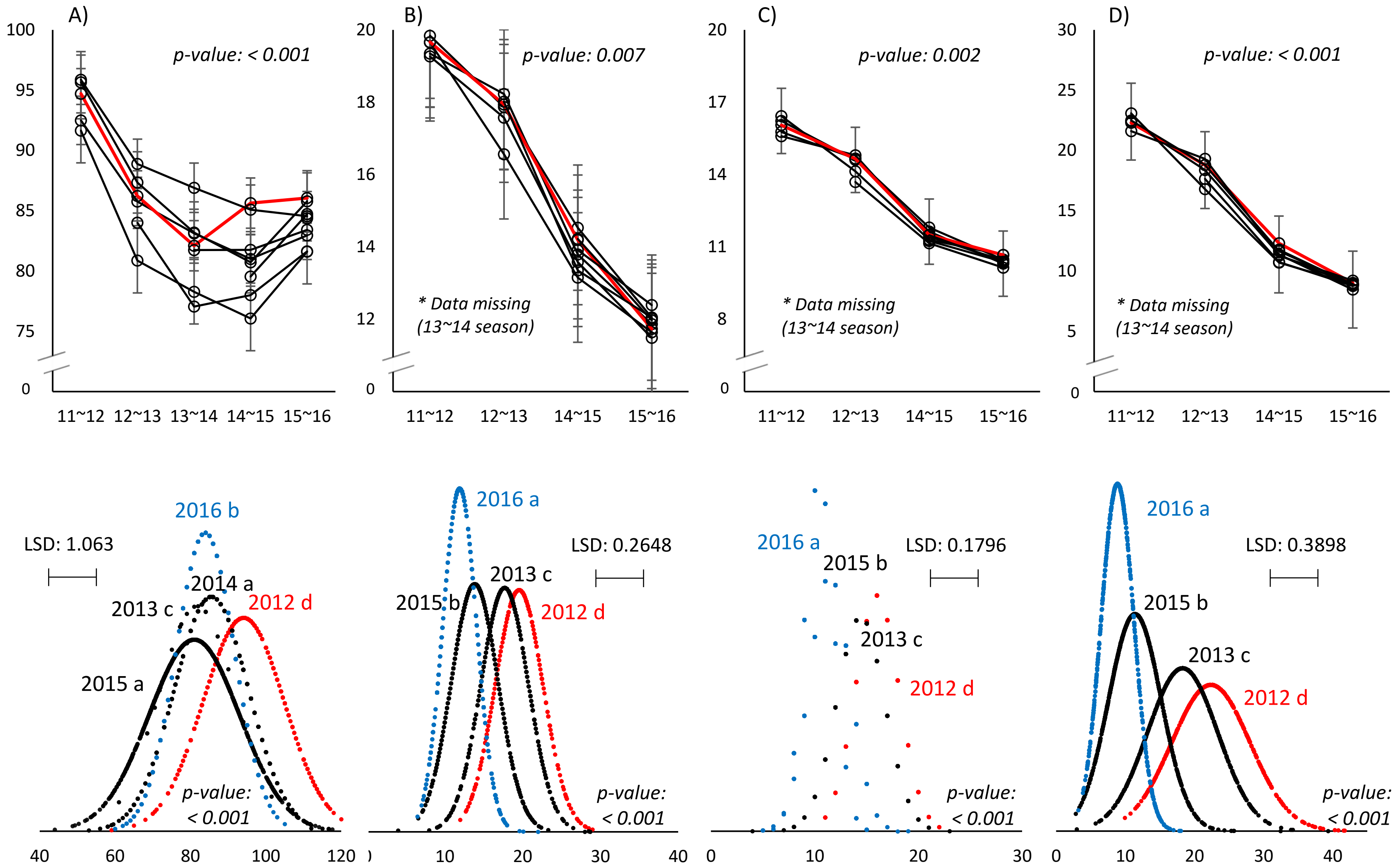


FIGURE 29. Genotype variance over year in CCPs (Top: genotype variance over year, Bottom: CCPs population variance over year) A) Plant height B) Flag leaf length C) Flag leaf width D) Flag leaf area. † top: red line indicates the oldest CCPs (CCPs- B), bottom: red line indicates 2011/2012 growing season and blue line indicates 2015/2016 growing season.

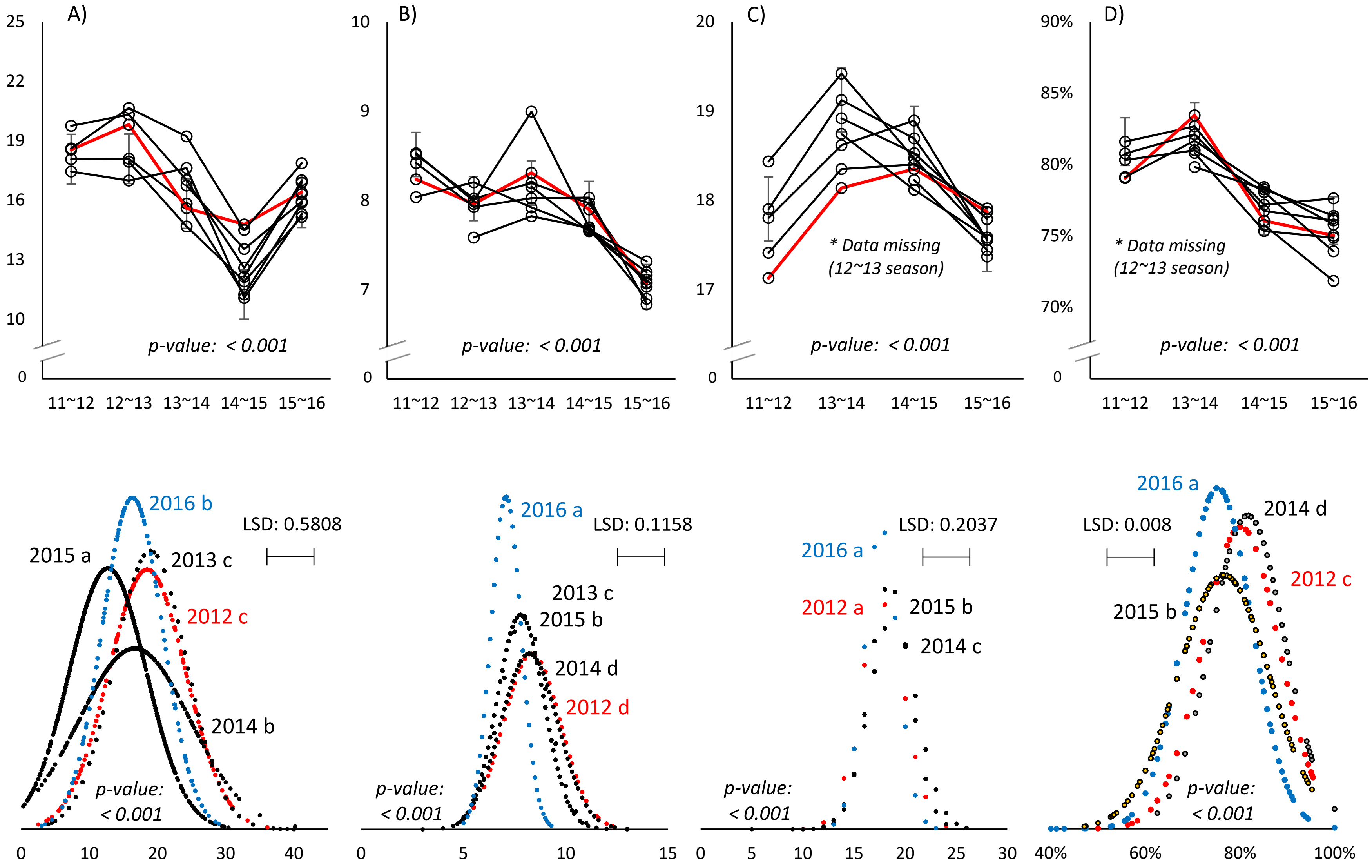


FIGURE 30. Genotype variance over year in CCPs cultivar (Top: genotype variance over year, Bottom: CCPs population variance over year) A) Distance between flag leaf and spike B) Spike length C) Number of spikelet D) Spike fertility. † top: red line indicates the oldest CCPs (CCPs- B). bottom: red line indicates 2011/2012 growing season and blue line indicates 2015/2016 growing season.

Previous data for growing season were analyzed to elucidate the G x E interaction in CCPs, all crucial traits showed interaction ($p<0.05$) between the genotype and year in different growing periods (2012~2016, 2012~2014 and 2015~2016) while yield and TKW showed no interaction ($p>0.05$) (Table 24, Figure 28, Figure 29, Figure 30). On the other hand, for varieties, there were only interactions ($p<0.05$) in vegetative traits and no interactions were shown in yield components. The genotypic value was usually defined to be equal to the expected phenotypic value at given environmental conditions. It means the genotypic value depends on the environmental growing conditions. What is more, the genotypic value also depends on the effect of interaction between genotype and environment. The simple statistical model describing the phenotypic value [P] of genotype [*G*] in environment [E], allowing for interaction [G x E] and experimental error [e] was already suggested in this paper as $V_P = V_G + V_E + V_{GE} + V_e$. This statistical model implies that the difference between the phenotypic value of genotype does not only depend on the main effects of the genotypes but also on the effect of their interactions with the environment (Bos and Caligari, 2008). When the phenotypic difference among genotypes is larger or smaller in one year than in another year, it can be indicated as G x E interaction. On the contrary, if genotypes respond in the same way and by the same amount, no or not much interaction will be found. In this research, only the yield and TKW showed no G x E interaction in CCPs. It indicates yield ($ton \cdot ha^{-1}$) and TKW (g) are more independent over years. For varieties, less G x E interactions were shown than CCPs. Actually, G x E interaction might contribute to the development of new varieties for breeders as G x E interaction implies a change of the ranking of genotypes between environments. When breeders exploit the change of ranks in different environments, new varieties could be effectively selected which can be adapted to certain environmental conditions such as low-input farming systems (Hoffmann *et al.,* 2009; Bos and Caligari, 2008). Therefore, CCPs could be better breeding materials than varieties. Through evolutionary breeding, new traits could be selected and exploited for further breeding programs particularly, under organic breeding program.

3.8. Adaptability and stability

The Finlay-Wilkinson regression model was applied in order to elucidate adaptability and stability. The environmental index was analyzed and the regression model of each CCP was suggested based on the environmental index. In this paper, a linear regression model was also suggested as $V_P = V_G + \beta_E + e$ to elucidate adaptability. If the regression coefficient (β) is high, it was suggested as high adaptability. Also, when each observed value is close to a regression line, it could indicate that the data pattern is stable. In this view, stability was calculated by *root-mean-*

TABLE 25. Adaptability and stability parameters: the regression coefficient for the slope of the regression line (β), and variance due to deviation from regression (s^{2}d) in Finlay-Wilkinson regression of Winter wheat under organic conditions from growing season 2012 to 2016

	Plant height (cm)			Flag leaf length (cm)			Spike fertility (%)			Yield (ton·ha^{-1})		
	C	β	s^{2}d	C	β	s^{2}d	C	β	s^{2}d	C	β	s^{2}d
B	86.2	0.72	7.76	15.3	0.96	2.16	0.8	0.98	0.06	2.58	0.87	0.64
A	84.2	0.78	7.51	14.7	0.99	2.21	0.8	1.00	0.06	2.66	0.81	0.15
G	81.0	0.99	7.89	15.1	0.99	2.21	0.8	0.99	0.06	2.67	1.15	0.56
D	85.6	0.98	7.93	15.0	0.98	2.28	0.8	0.98	0.06	2.58	1.02	0.40
E	87.4	0.77	8.58	14.5	0.98	2.20	0.8	1.08	0.06	2.62	1.14	0.44
H	81.4	0.97	6.62	15.1	1.04	1.60	0.8	0.94	0.06	2.83	0.60	0.39
I	83.5	0.39	7.51	16.0	1.33	2.21	0.7	1.19	0.06	2.79	1.14	0.24
J	85.8	1.31	8.29	15.1	0.89	2.09	0.7	4.66	0.06	2.74	1.35	1.37
C	79.4	1.11	5.67	15.3	1.07	2.34	0.7	1.07	0.06	2.42	1.19	0.41
K	66.6	0.81	6.44	11.4	-0.03	1.64	0.8	-2.30	0.06	2.01	0.51	1.38

† β indicates adaptability across the environments and s^{2}d indicates stability of data pattern

square error (RMSE) which indicates the high value of *RMSE* is unstable data pattern. Even though genotype performances of most traits usually have a trend to decrease over year in CCPs, its adaptability showed a positive regression model in all CCPs (Table 25). It suggests that CCPs have wider adaptation across a range of environments. *Julius (K)* showed a negative adaptability in the flag leaf length and spike fertility and a low stability in yield. It means that *Julius (K)* has a narrow adaption across a wide range of environments and it might result in low performances

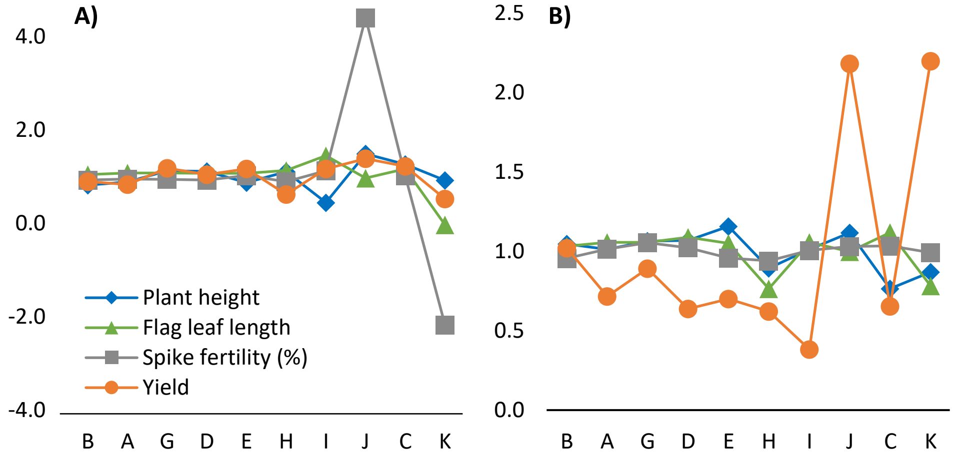


FIGURE 31. The relative mean value for A) adaptability B) stability in Finlay-Wilkinson regression of winter-type wheat under organic conditions from growing season 2012 to 2016. † The higher value of stability indicates less stable data pattern.

under environmental changes. On the other hand, *Naturastar (C)* showed a higher adaptability and stability. There were interactions between Genotype and Year in most of the traits of CCPs. It means that genotype differences in phenotypic responses are dependent on the environments. Therefore, in CCPs, a simple comparison of genotype performances over the years might not be an appropriate approach to evaluate the performance of CCPs. On the contrary, it would be a more important evaluation to assess its adaptability and stability across a wide range of environments. Only one final result of adaptability and stability were calculated using all data from the first and last season. It means that multiple comparisons (i.e. LSD or Bonferroni) regarding accessions is not possible. Instead, the pattern of adaptability and stability over years was able to be observed. Therefore, in order to clearly compare the data pattern of each accessions over the years, a relative mean of adaptability and stability was calculated. For adaptability, all accessions showed a similar pattern, while *CCP-J* and *Julius (K)* showed a much higher or less relative mean of adaptability with respect to spike fertility (Figure 31 A). On the other hand, for stability, yield showed a different data pattern unlike other three traits over the years. Also, *CCP-J* and *Julius (K)* showed a much higher or less relative mean of stability in yield (Figure 31 B).

4. Discussion

First of all, the germination test about wheat seeds harvested in 2015 was conducted according to the ISTA regulation to elucidate that each accession has different a germination capacity. In this germination test, the seed dormancy was not considered. So, any seed treatments such as *pre·chilling* were not performed. All accessions a germination rate of more than 90% but there was no significant difference (p=0.459) among accessions (Appendix 5). Abnormal and small seedlings were also investigated in order to present a minimum germination capacity, which possibly results in low seedling emergence under poor environmental conditions. However, those seedlings also showed no significant differences (p>0.05). Even though all accessions have a similar germination ability, it exhibited different seedling emergences in the real field (Table 12). There was no correlation (p=0.298) between the germination rate (%) under the optimum condition and the seedling emergence in the experimental field. Also, the minimum germination ability did not show a correlation (p=0.987) with the seedling emergence (data was not shown). Therefore, it can be inferred that early seedling emergence of winter wheat is more affected by environmental factors, not its own germination capacity as paper germination test provides constant temperature and humidity (for wheat, 20°C, 65% Humidity). Also, this result indicates that all accessions have the same seed quality with respect to the vigor. Therefore, the differences of early vigor among accessions result from genotype variance of each accession or different plot conditions.

In the early growing stage, no correlation was found between the early seedling emergence and tillering in CCPs, whereas varieties showed a positive correlation (Figure 17). It can be indicated that tillering in varieties is much more affected by early seedling numbers than CCPs. Under high environmental variability, seedling emergence in the growing field might be the most vulnerable trait. Therefore, it can also be suggested that tillering in CCPs are more resilient against environmental variability than in varieties in the early growing stage. In addition, two varieties showed a lower reduced rate (%) of tillering than CCPs. Actually, the low reduced rate (%) of tillering indicates that most of the early tillers became possible fertile tillers. It means that the two varieties had a lower buffering capacity of tillering than CCPs. When environmental conditions are unpredictable, early tillering will be dramatically reduced and it might result in low fertile tillering if early tillering has a low buffering capacity against environmental variability. In case of CCPs, even though the reduced rate (%) of tillering was much higher than the varieties, the total number of tillers was also higher. Therefore, it can be suggested that CCPs have a higher buffering capacity of tillering against environmental variability than in varieties.

The early ground cover rate (%) also showed a significant difference among accessions. However, except for Julius (K) which showed the lowest cover rate (%), it exhibited a similar cover rate (%). On the other hand, there was no significant difference among all accessions in the late ground cover rate (%) (Table 12). In late growing stage, wheat leaves are fully developed and it might show a similar cover rate (%), even though each accession has a different number of plants. It means that the early ground cover rate (%) needs to be considered as a more crucial factor that affects the yield potential as the late ground cover rate (%) does not have any distinct characteristics among accessions. Also, the ground cover rate did not show any positive effects on the weed density (%). It was expected that a high ground cover rate (%) will result in a high weed suppression but no regression was found between the ground cover rate and weed population. In this experimental field, however, *Vicia sativa* was the most predominate weed species which covers the wheat with many branches. The weed density was usually based on the appearance of *Vicia sativa.* Therefore, this weed appearance is not able to be comparable with that of other weed species to define weed competitiveness in this field. Even though the ground cover rate (%) was high, the weed suppression might be less evaluated because of the widespread shape of the *Vicia sativa.* Weed suppression cannot be estimated with only one single characteristic. It is based on the interaction between various favorable characteristics such as crop physiology and allelopathy (Donner *et al.,* 2006). In the experimental field, however, there were various weed species observed during the early growing stage but those were too different from each other to evaluate direct negative influence on wheat growth. For example, *Vicia sativa*, clover and flixweed were mostly observed in the experimental field but clover and flixweed did not have high negative influences on wheat growth and were also easy to control during all growing seasons. What is more, clover is a leguminous crop which shows nitrogen-fixing and it might result in higher nitrogen use efficiency in wheat. On the contrary, *Vicia sativa* directly affected the wheat growth as it covered the whole plant and pulled it down. So far, in terms of crops, favorable characteristics against weeds have been continuously studied. In terms of weeds, however, it will be indispensable to understand physiological and morphological characteristics of weed species in the future. Simply elucidating weed competitiveness on the basis of crop characteristics might not fully explain weed suppression as there are various weed species which have different physiological and morphological characteristics. Actually, in the regression model between weed density and flag leaf length, *CCP-I* and *CCP-J* showed no decreases (Figure 22 B). Those accessions could be considered as strong or weak competitiveness. In order to elucidate weed competitiveness in accessions, further research will be necessary to investigate the interaction among genotype(s), weed species and farming systems.

The ground cover rate (%) also affected the plant height and flag leaf length in CCPs. In multiple regression models, both for the plant height and flag leaf length, the coefficient (β_1) of the ground cover rate was much larger than other variables (plant height; 41.85 x_{1i}, flag leaf length; 5.39 x_{1i}). Therefore, it can be inferred that the ground cover rate is the most important factor affecting the plant height and flag leaf length in CCPs. It also indicates that maintaining early vigor in growing field is indispensable to increase the yield potential. However, for plant height, when the ground cover increased, *CCP-H* and *Julius (K)* decreased (Figure 21 A). Also, when the flag leaf length increased, *CCP-H* decreased (Figure 21 C). Therefore, it can be inferred that *CCP-H* has an even nutrient availability throughout all plant tissues, not highly translocated t only certain parts of tissues. What is more, *CCP-H* exhibited the highest greenness (%) (Table 15). This result might result from the relatively low weed density. *CCP-H* was under the lower weed pressures than other accessions (Figure 15) and also the lowest plot damage by weeds (Table 10). Also, when the ground cover rate (%) increased, the oldest CCPs, *CCP-A* and *CCP-B* decreased in flag leaf length, whereas other accessions showed the opposite result (Figure 22 A). It might need further researches to elucidate the relationship between CCPs cycling times and certain traits because only one regression model might not enough to explain certain phenomenon.

CCP-J showed the highest TKW (g) but the lowest greenness (%) of flag leaf (Table 15). Therefore, it can be inferred that when the greenness (%) of the flag leaf is low in wheat, the nutrients are highly located to the grains from the leaf. Nitrogen is important for leaf growth and it provides the rich green color of the leaf, yellowing (chlorosis) will be observed under the condition of N deficiency. In organic farming systems, however, genotypes that show an early uptake of nitrogen and translocation to the grain might be more adapted to different environments as there is a shortage of nitrogen in the late growing stage (Donner *et al.,* 2006). It means that in low-input farming systems, it is essential that the nitrogen uptake into the grain will be improved to maintain yield potential which relies on (1) total N uptake from the soil and (2) translocation from leaf to the grain (Wolfe *et al.,* 2008). It can be inferred that genotypes which have been developed under organic conditions intend to uptake nitrogen from the soil and translocate nutrients to grains earlier than genotypes in conventional farming systems. Therefore, in CCPs populations, the low green color on the flag leaf might indicate a high nutrient translocation to the grain. However, although *CCP-H* exhibited the highest greenness (%), it also showed a relatively high TKW (g). What is more, as mentioned earlier, *CCP-H* showed lower weed pressures. Therefore, potential yield or grain quality cannot be estimated only by the greenness (%) of the flag leaf as a high greenness of the flag leaf might also result from (1) a higher assimilation efficiency on leaves or (2) lower competition with weeds. In this research, only one greenness (%) was measured, so it is most likely not possible to elucidate the threshold to distinguish translocation, assimilation or weed effects. However, it is suggested that maintaining the greenness on the flag leaf in the early growing stage might be an important factor in increasing the yield potential of wheat production.

In the multiple regression model of the number of spikelets, the coefficient (β_2) of the distance between the flag leaf and spike showed a negative effect ($\beta_2 = -0.01$). The value was relatively small and it might less affect the number of spikelets. However, this result can argue against the common theory regarding the advantages of the distance between the flag leaf and spike. Previous research suggest that a longer distance between the flag leaf and spike can reduce the transfer of spores from the leaves to the spike by rain drops and can also minimize spike infections (Reviewed by Asif *et al,* 2014). In this research, however, the relationship between the distance and the actual spike diseases (%) was not found. In CCPs populations, the plant height is generally heterogeneous and it can be inferred that spores in tall plants can move to adjacent short plants and eventually infect the spike. In organic breeding, theoretically, the distance between the flag leaf and spike is an advisable trait to prevent spike diseases. However, at the same time, it might always not be a crucial breeding goal as there are too many variables to simply define the advantages of a long distance between the leaf and spike in CCPs.

In this research, various phenotypic performances of each trait were observed in different accessions. Based on G x Y interactions, the phenotypic performances showed significant differences over years. For the flag area (length and width), the phenotypic performance of each CCP showed declining patterns over years and the entire CCPs population of each year also showed the same declining pattern over years (Figure 28). When the main effect was analyzed, the F-value of flag leaf characteristics (length and width) about the year effect was much higher than the F-value of other traits (Table 24). It indicates that flag leaf characteristics have a high year effect which means that the phenotypic performance is more dependent on environments. The phenotypic performance might be good in one season but it also might show a low performance in another season. Therefore, it can be inferred that the phenotypic performance of flag leaf characteristics is not a stable trait under environmental variability. On the contrary, other traits showed a relatively low year effect compared to the flag leaf. Also, when comparing the (yearly) relative mean over year, the plant height, the distance from flag leaf to spike and the number of spikelet showed a respectively similar phenotypic performance (Figure 23). Even though some relative mean graphs did not look like a similar pattern, if previous specific seasons showing poor environmental conditions such as high weed pressures or flooding are removed, it showed similar pattern of relative means. It indicates that late and reproductive traits are more stable than early agronomic traits. Actually, the plant height, the distance from flag leaf to spike and the yield components except for TKW (g) showed a relatively higher heritability than early agronomic traits (Table 22). Therefore, it can be inferred that environmental factors highly affect agronomic traits in the early growing stage. Late and reproductive traits might be genetically more stable for survival and propagation during the entire evolutionary process. Also, for varieties, the G x Y interaction was mostly not found in yield components (Table 24). It is known that if all genotypes respond in more or less the same way and by the same amount in the different environmental conditions which cause the phenotypic variance due to some kind of biotic and abiotic stress, it is considered as no or not that much interaction (reviewed by Maliepaard, 2014). It means that two varieties showed the same phenotypic variance of yield components in different environmental conditions. Under the organic breeding program, it might be important to develop accessions that are able to cope with various abiotic and biotic stress factors as compared to conventional breeding, organic breeding has fewer tools to influence the growing environment to accommodate crops (*Kokare* et al., 2014). However, in case of G x Y interaction, it might not be effective for accessions to be evaluated in different years and affect the selection of new type of accessions to be developed. Therefore, in organic breeding, it can be suggested that two varieties are less efficient breeding materials than CCPs in this research. Furthermore, all accessions did not show any G x Y interaction over year in the final yield (ton·ha^{-1}) (Table 24). Also, the h^2 of the yield was much lower than the yield components in CCPs (Table 22). Due to the low h^2, the yield is more affected by environmental factors than the genotype. What is more, the yield (ton·ha^{-1}) differences among CCPs are equal from one year to another under no G x Y interaction (Figure 28 B). As a yield component, TKW (g) also showed the same result as the yield (Figure 28 A). Therefore, it can be suggested that in CCPs, genotype performance of the yield (ton·ha^{-1}) was not highly shown over years and the yield might be more affected by environmental conditions.

However, genotype differences over years cannot fully explain the actual performance of CCPs as environmental conditions are changing. Therefore, in this research, it is suggested that, in CCPs, the adaptation or stability of genotypes across a wide range of environments might be a more crucial element. it was shown that CCPs and *Naturastar (C)* showed a higher adaptability and stability than *Julius (K)* (Table 25). Adaptability indicates the flexibility of a genotype in its response to improved environments and stability indicates little variation across environments (reviewed by Maliepaard, 2014). If a genotype is performing well across a wide range of different environments, it is considered as a wide adaptation. In the Finlay-Wilkinson regression model, all CCPs and *Naturastar (C)* showed a positive adaptability. Therefore, it can be suggested that all CCPs and *Naturastar (C)* have a wide adaptation across a wide range of different environments. For *Julius (K)*, it showed a much lower adaptability and it even showed a negative value in the flag leaf length and spike fertility (%). In this growing season, Julius *(K)* almost failed to grow in the experimental field. It means that *Julius (K)* is not able to adapt itself to low-input farming systems. In other words, *Julius (K)* has a narrow adaptation under organic farming systems. Stability was calculated as a deviation from Finlay-Wilkinson regression model. A low stability value indicates little variation over environments or years as the closer the observed data is to the regression line, the smaller the deviation between the regression and real values. Even though the genotype performance in CCPs showed some decreasing or fluctuating pattern in various traits over years (Figure 29, Figure 30) and stability were shown over years. It indicates that CCPs have a wide adaptation across a wide of environments and improving CCPs could be a new breeding paradigm in the future.

5. Recommendations

First of all, this research project has been based on the genotype and year interaction. However, in order to elucidate the genotype performance precisely, the genotype, year and location (G x Y X L) interaction should be suggested. In this research, only one experimental factor (genotype) was not enough to elucidate each genotype performance. As this G x E interaction model, heritability can be calculated in order to elucidate genotype variance of accessions. Also, adding a new experimental factor such as farming systems would be more beneficial to investigate how the genotype performance is different under different farming systems. In terms of METs, if G x Y x L x F interaction (F: farming system) is conducted, it would discover more possibilities of accessions as a new genetic material. Second, more replicates might be required to increase statistical power. Only 3 replicates (i.e. blocks) were used in this research but more replicates would be necessary in the future. Also, more pure line varieties would be required to be added for further researches. Only two varieties compared 9 CCPs and it was unbalanced to analyze G x E interaction. Third, uniform data collection is highly required. Every year, different investigator conducted the consecutive research. So, according to his or her interests, new data collection was added but at the same time, other data collection was missing. This research project is crucially based on the analysis of continuous data patterns over years. Therefore, it is important to establish a database which contains previous data collections so that a new investigator for the next seasons can follow the same data collection. Finally, to set up software tools to obtain quantitative data would be also required. As different investigators conducted this research project, variation for observed values might be substantially high. Some data based on personal phenotyping such as the ground cover rate, weed density and the degree of green on leaves might not be compared with previous years because of wide data variation. Therefore, applying software tools which are able to provide a standard protocol is highly required. In this research, MATLAB and image software program were used on a trial basis. In the future, if various software tools are being developed, more precise quantitative data could be obtained.

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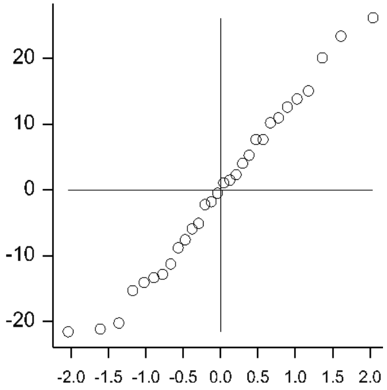
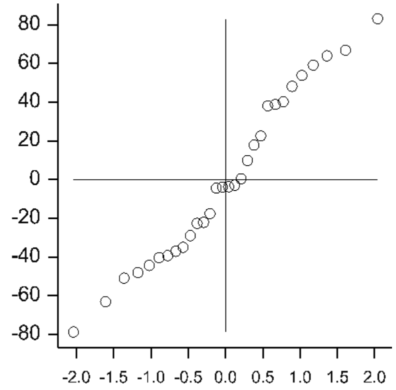
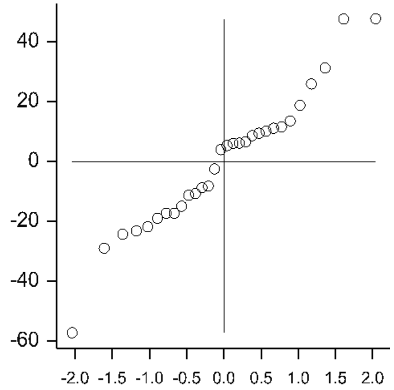
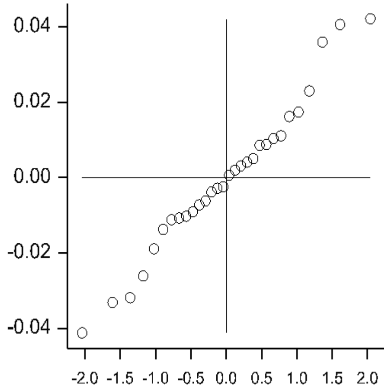
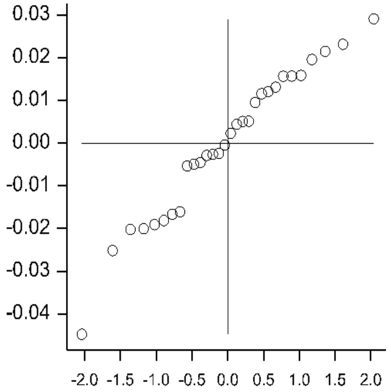
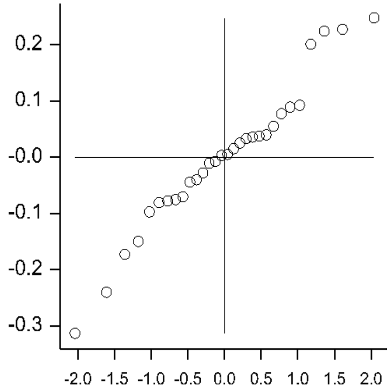
Appendix

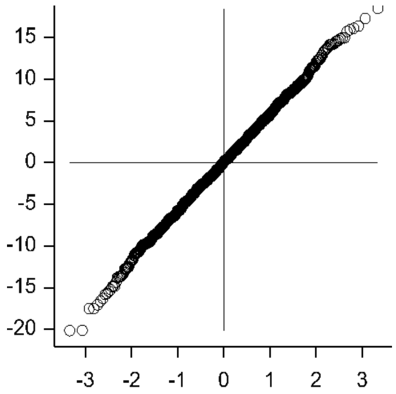
APPENDIX 1. The analysis of data normality of early vegetative traits in growing season 2015/2016

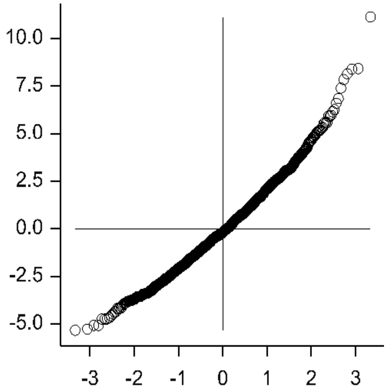
	Early seedling emergence	Early tillering	Late tillering
Data set	Plot mean data	Plot mean data	Plot mean data
Normality	Normalized	Normalized	Normalized
Multiple comparisons	Fisher's protected LSD	Fisher's protected LSD	Fisher's protected LSD
			

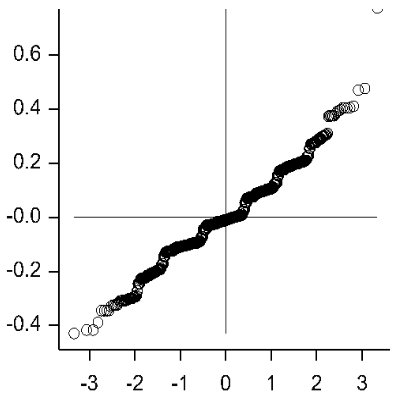
	Early ground cover rate	Late ground cover rate	Weed density
Data set	Plot mean data	Plot mean data	Plot mean data
Normality	Normalized	*non-parametric*	*non-parametric*
Multiple comparison	Fisher's protected LSD	Bonferroni correction	Bonferroni correction
			

APPENDIX 2. The analysis of data normality of early vegetative traits in growing season 2015/2016

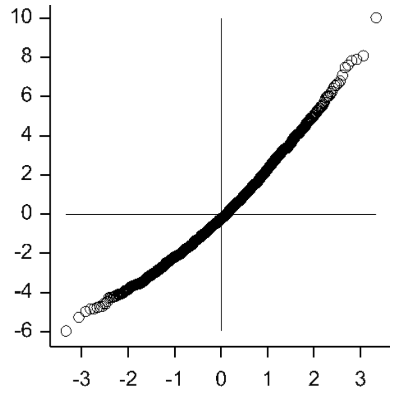
	Plant height	Flag leaf length	Flag leaf width
Data set	Raw data	Raw data	Raw data
Normality	Normalized	Normalized	Normalized
Multiple comparisons	Fisher’s protected LSD	Fisher’s protected LSD	Fisher’s protected LSD

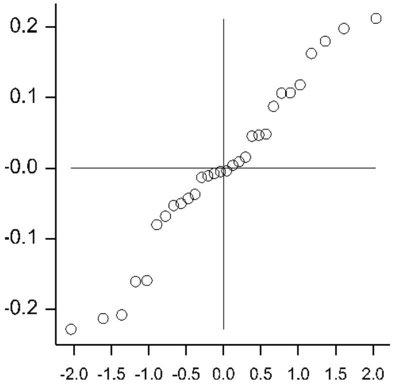


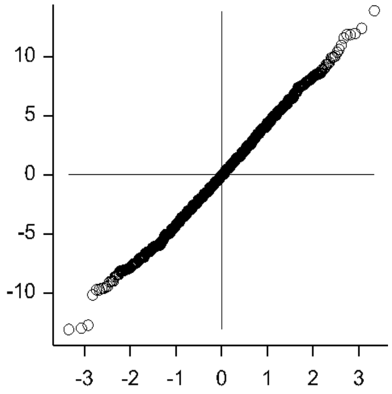




	Flag leaf area	Flag leaf greenness	Distance between flag leaf and spike
Data set	Raw data	Plot mean data	Raw data
Normality	Normalized	non-parametric	Normalized
Multiple comparison	Fisher’s protected LSD	Bonferroni correction	Fisher’s protected LSD

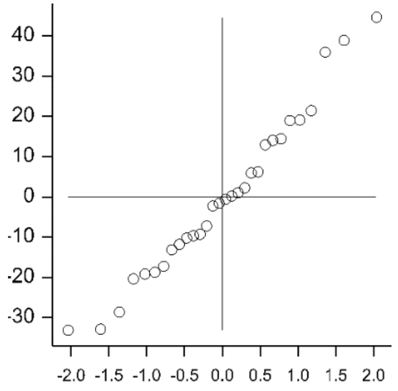


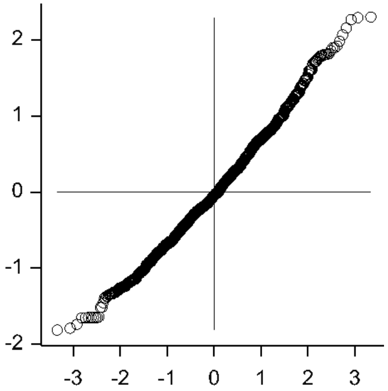


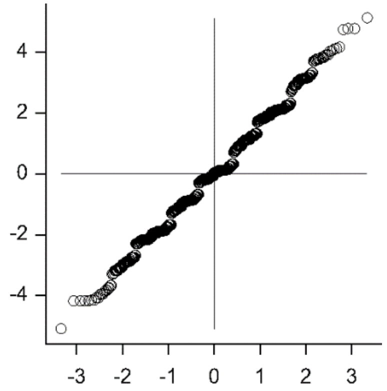


APPENDIX 3. The analysis of data normality of yield components in growing season 2015/2016

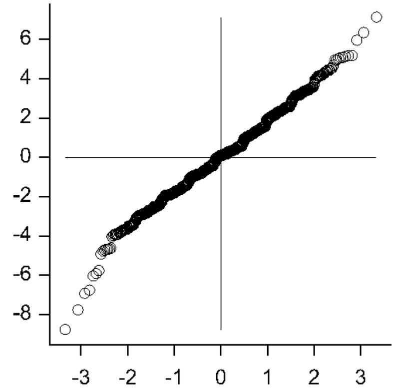
	Number of fertile tillers	Spike length	Number of spikelet per spike
Data set	Plot mean data	Raw data	Raw data
Normality	Normalized	Normalized	Normalized
Multiple comparisons	Fisher's protected LSD	Fisher's protected LSD	Fisher's protected LSD

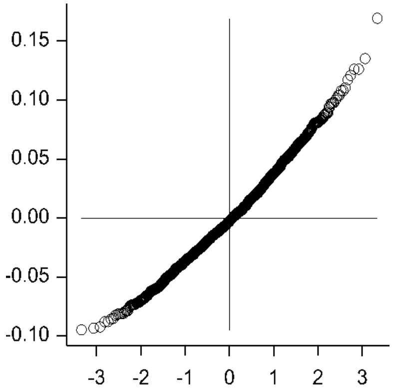


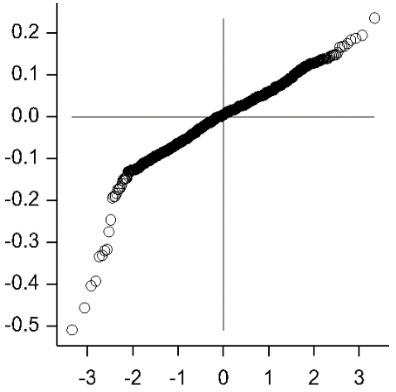




	Number of fertile spikelet per spike	Spikelet density per spike	Spike fertility
Data set	Raw data	Raw data	Raw data
Normality	non-parametric	Normalized	non-parametric
Multiple comparison	Bonferroni correction	Fisher's protected LSD	Bonferroni correction

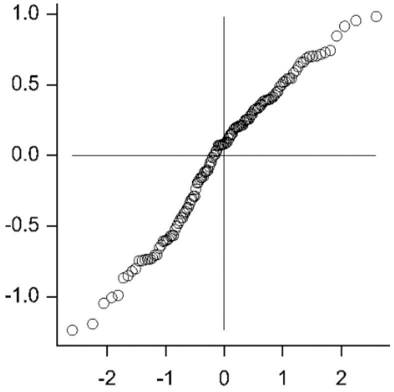


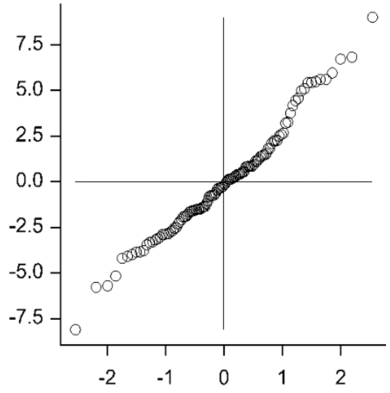




APPENDIX 4. The analysis of data normality of yield and TKW in growing season 2015/2016

	Yield (ton·ha^{-1})	TKW (g)
Data set	Raw data	Raw data
Normality	Normalized	Normalized
Multiple comparisons	Fisher's protected LSD	Fisher's protected LSD

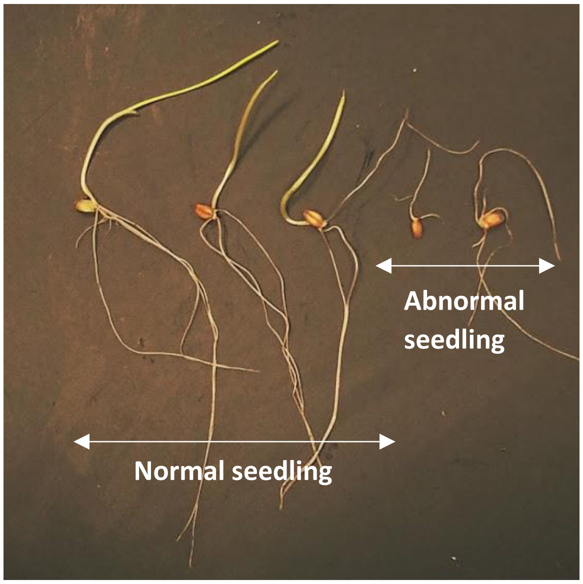


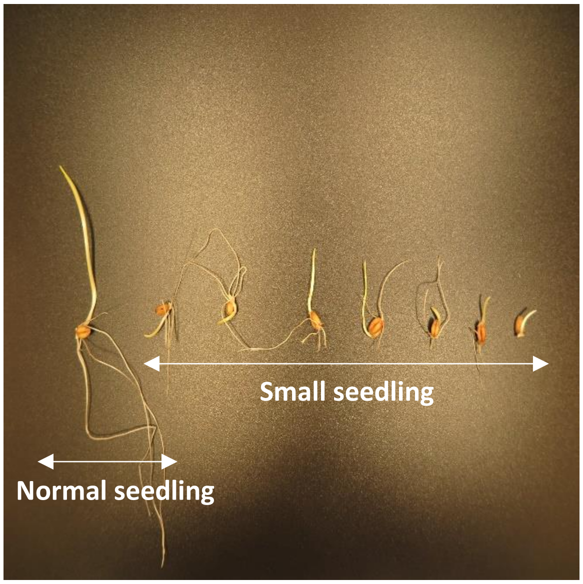


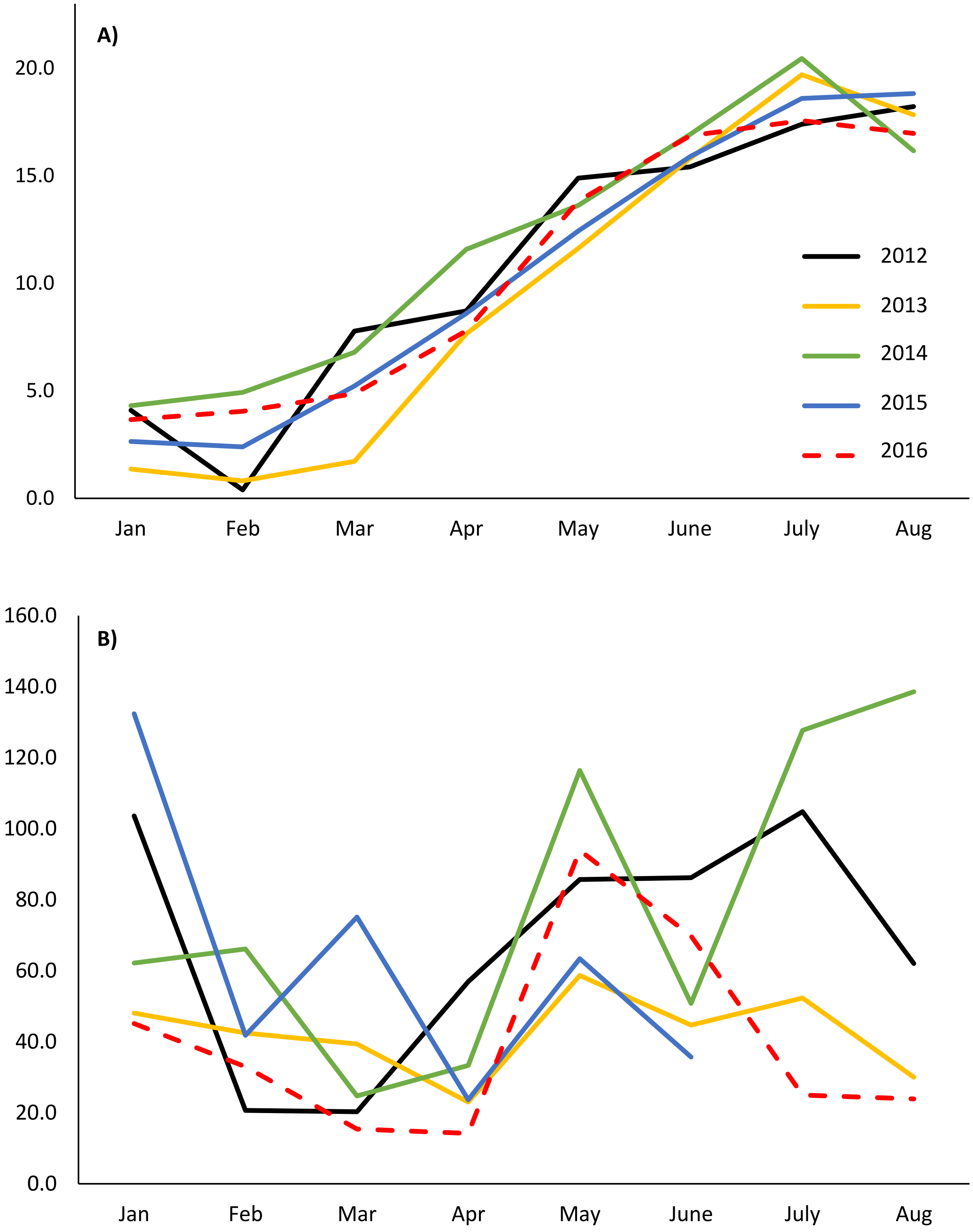
Appendix 5. **Germination rate (%) of all accessions sown in 2015/2016**

Cycling years	Accessions	1st counting (%)	Final Germination (%)	Abnormal seedling (%)	Small seedling (%)	Minimum germination (%)[1]
9	B	87.3	98.0	0.7	3.3	94.0
9	A	90.7	96.7	0.7	5.3	90.7
8	G	82.7	98.7	0.7	8.7	89.3
7	D	81.3	95.3	1.3	10.7	83.3
6	E	84.0	94.0	0.7	4.6	88.7
5	H	72.0	92.0	1.3	9.4	81.3
4	I	85.3	94.0	0.7	4.0	89.3
3	J	84.0	96.7	1.3	8.0	87.3
2	L	90.7	97.3	1.3	3.3	92.7
-	C	82.7	95.3	0.0	10.0	85.3
-	K	82.7	96.0	0.0	11.3	84.7
	F-value	0.71	1.02	0.98	0.71	0.71
	p-value	0.708	0.459	0.492	0.703	0.704

[1] Minimum germination (%) = Final germination (%) - Abnormal seedling (%) - Small seedling (%)







APPENDIX 6. **Climate data pattern (2012 to 2016)** A) Mean temperature (°C) B) Precipitation (mm) from January to August.

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