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Published version:

DOI:10.1016/j.eja.2015.09.006

UNIVERSITÀ DEGLI STUDI DI TORINO

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Maize grain yield enhancement through high plant density cultivation with different inter-row and intra-row spacings.

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Abstract

Increasing the maize plant population has undergone a constant evolution over the years, with the purpose of enhancing the crop yield. Field trials, in which 2 different experiments were considered, have been conducted in NW Italy in order to analyze the yield benefit of high plant density on recent hybrids, considering both intra-row and inter-row plant spacings. The first experiment, which was carried out during the 2013 and 2014 season, evaluated the effects of 4 growing plant densities (from 7.5-to 12 plants m^{-2}) combined with 2 row widths (the traditional 0.75 m and a narrow inter-row spacing of 0.50 m) on plant architecture-and grain yield. Two hybrids, with different ear developments (fixed and flex), were taken into account. The higher plant density led to a decrease in the stalk area (-20%), leaf greenness (-5.2%) and cob length (-10.8%). It also negatively affected the kernel weight (-7.1%) and the number of kernels per row (-10%). The grain yield only increased significantly, for both hybrids (+7.4%), if a density of 10.5 plants m^{-2} was reached when the inter-row spacing was reduced (0.50 m). In the second study, an innovative system (narrow inter-row spacing combined with a plant population of about 10.5 plants m^{-2}) was compared with a standard planting system (7.5 plants m^{-2} sown on 0.75 m wide rows), considering 32 different production situations (PS) over 4 growing seasons (2011-2014). This study has confirmed that even though the single plant yield potential was reduced in terms of ear weight and thousand kernels weight by 18% and 6% respectively for a high planting density, the final grain yield increased on 90% of the PS with an average gain of 11.7%. This work has proved that, in the conditions in which the experiments were conducted, a high planting density of up to 10.5 plants m^{-2} can lead to a significant yield increase, but only when it is combined with narrow inter-row spacing. These conditions increase plant stresses, and modify plant morphology and development to the detriment of the single plant yield. However, the lower yield per plant is fully

compensated by the higher plant population.

Keywords: seeding rate, uniform planting pattern, plant traits, kernel traits.

Abbreviations: ECB, European Corn Borer; ESQ, ears per square meter; HI, harvest index; HiD, innovative high planting density system; HNT, hydro N-tester; KD, kernel depth; KL, kernel length; KPR, kernels per row; KR, kernel row; KSQ, kernels per square meter; KV, kernel volume; N, nitrogen; NIS, narrow inter-row spacing; PS, production situation; RR, relative ratio; SIS, standard inter-row spacing; StD, standard density planting system; TKW, thousand kernels weight; TW, test weight.

1. Introduction

Increasing the population density of plants is an agronomical practice that has continuously been studied for maize crops. This crop technique has evolved and will continue to evolve over the years and it is the agronomic management factor that has changed the most over the past six decades (Tollenaar, 1992). After the introduction of the first hybrids, farmers started to steadily increase the plant density, at an average rate of 0.3 plants m^{-2} yr^{-1}. In the US Corn Belt of the 1930s, the mean population density was 3 plants m^{-2}, while it was 4 plants m^{-2} in the 1960s and 6 plants m^{-2} in the 1980s (Duvick, 2005). Nowadays, the average density in the USA, where maize cultivation is intense, is around 8 plants m^{-2} (Li et al., 2015), whereas in the EU, where the pedo-climatic conditions are more heterogeneous across countries, it can vary from 6 to 8 plants m^{-2} for medium-late maturing hybrids in fertile growing areas. On both continents, in which this crop is cultivated intensively, the most common inter-row spacing for the current planting density is approximately 0.70 - 0.75 m in order to facilitate inter-row tillage ~~(~~Sharratt and McWilliams, 2005). The main purpose of increasing the plant density is to enhance maize yield in terms of grain or biomass, thus making the crop system more efficient and competitive per area unit. In the absence of biotic or abiotic stresses, grain yield is related to the amount of solar radiation intercepted by the crop, and the use of a high density population, with an earlier canopy closure, maximizes the leaf area index (Cox and Cherney, 2001)

The crop yield potential could be divided into three genotype components. The first one is the yield potential per plant, which, over the last 70 years, has remained stable for non-stressed plants under very low planting densities (1 plant m^{-2}) (Duvick et al., 2004a; Sangoi et al., 2002). The second component is the tolerance to various abiotic and biotic stresses, and the third one is responsiveness to inputs (Tokatlidis and Koutroubas, 2004).

In the late 1930s, the average grain yield in the United States was around 2 t ha^{-1} (Duvick et al., 2004a). After this, the mean yield gain was increased each year by 115 kg ha^{-1} until the 1990s, when the yield expectation was around 8.8 t ha^{-1}. This occurred thanks to the positive interaction between enhancements in cultural practices and plant breeding, which improved components 2 and 3 of the crop yield potential (Duvick, 2005). Continuous changes in plant architecture have led to an increase in the efficiency of grain production under stresses for high planting densities, because of higher intra-specific competition, unfavorable weather, or low soil fertility (Duvick et al., 2004a).

The shoot and seed density per area unit of modern maize hybrids is theoretically the same, since these plants have lost their tillering ability (Duvick et al., 2004b), to the advantage of a higher harvest index (HI) on the single plant. Thus, the only way that maize plants can moderately compensate for planting density changes on the final yield is through an adaptation of ear development. A flex ear hybrid adjusts its ear growth according to the conditions that are encountered in the field, by modifying the number of kernels per ear that reach full maturity. This feature allows, for example, to better compensate for plant vacancies and hence preserve the yield if harsh field conditions occur (Mascagni and Bell, 2004). On the other hand, a fixed ear development keeps the total number of kernels per ear relatively steady, regardless of the environmental conditions. Maize is one of the herbaceous species that offers the highest grain yield potential, yet it is one of the most sensitive crops to variations in plant density (Vega et al., 2001). Modern hybrids can generally withstand higher population densities, because they can support stressful environmental conditions caused by a higher intra-specific competition more easily, and this results in a lower incidence of barren plants and reduced stem lodging (Tollenaar, 1989; Widdicombe and Thelen, 2002). In order to overcome the problems of high density planting, these hybrids are characterized by more upright and shorter leaves, and the synchrony between the differentiation of the female and male florets is higher (Sangoi, 2000), thus limiting barrenness. However, in order to place the crop in the best growing conditions possible, even for high plant populations, the planting pattern has to be re-thought with a reduction in the inter-row spacing and a more balanced equidistance. Plants spaced more uniformly in fact compete minimally for the main growing factors. Among these factors, the one that is affected most is light, and this is followed by nutrients and water (Li et al., 2015; Sharratt and McWilliams, 2005). According to this line of reasoning, an increase in planting density, obtained by reducing the within row plant spacing, could be a limiting factor. In fact, if plants are sown too close to each other in the same row, their full yield potential could be restrained.

The objective of this work was to evaluate the effects of plant density on maize yield and on the ear and plant morphology of hybrids with different ear development for full-season varieties under irrigation conditions. The interaction between the plant density, considering the reference plant population generally applied in temperate areas and different increments of up to 40%, and inter-row spacing, has been considered. Since the plant arrangement is one of the most important practices employed to increase maize yield (Okumura et al., 2014), ~~T~~this information could help to identify the best actual planting pattern for intensive high-yield maize cultivation in temperate areas, which could contribute significantly to an improvement in the competitiveness of this cereal.

2. Materials and methods

2.1 Experimental sites and agronomical management

The study on the effects of different planting densities on the morphological development of plants and ears and grain yield was performed over four growing seasons, from 2011 to 2014, and it involved two different experiments conducted in the same agricultural area in the Piedmont region in North West of Italy, characterized by a humid subtropical climate according to the Köppen climate classification (Peel, 2011).

2.1.1 Experiment 1

Field trials were conducted in the 2013 and 2014 growing seasons at Buriasco, in the North West of Italy (44°51’53’’ N, 7°26’21’’ E), an area that is characterized by a deep and fertile loamy soil (40% sand, 46% silt, 15% clay, Typic Hapludalfs, USDA classification) and a medium-high content of organic matter (3.2%) with a balanced C/N ratio (11.0). The N, P_2O_5 (available) and K_2O (exchangeable) soil content were respectively high (1.7 g kg^{-1}), medium (23 ppm) and low (18 ppm). The soil pH was sub-acid (6.3) and the Cation Exchange Capacity was medium (16.3 meq 100 g^{-1}).

The compared treatments were a factorial combination of:

- 2 inter-row spacings:
    - 0.75 m wide, standard inter-row spacing (SIS), representing the reference spacing for the maize crop system;
    - 0.5 m wide, narrow inter-row spacing (NIS)
- 4 planting densities:
    - D1: 7.5 plants m^{-2} (reference planting density)
    - D2: 9 plants m^{-2}
    - D3: 10.5 plants m^{-2}

    - D4: 12 plants m^{-2}
- 2 hybrids characterized by a different ear development according to the environmental conditions that occur during the growing season:
    - Syngenta NX7234, FAO maturity class 500; 127 relative days to maturity, characterized by a fixed ear development.
    - KWS Korimbos, FAO maturity class 500; 125 relative days to maturity, with a flex ear development.

The experiment was carried out according to a split plot design: the inter-row space and hybrid were considered as the main factors, whereas the planting density was considered as a sub-plot factor, with 3 randomized replicates. The whole field was planted at a maximum planting density of 12 plants m^{-2}, and then each plot was thinned at the four leaf stage (growth stage GS 14) (Lancashire et al., 1991) to adjust the plant population to the desired levels. The plant density was then checked again by counting the plants from GS 65 to GS 75. The sub-plots for the 0.5 m inter-row spacing were 10 m. long and 12 rows wide, and were 8 rows wide for the 0.75 m inter-spacing. The plot alleys, which were orthogonal to the maize rows, were 1 m wide.

The previous crop was maize each year. Mechanical ~~P~~planting was carried out on April 17th and March 17th, respectively, for 2013 and 2014, after an autumn 0.3 m deep ploughing, followed by disk harrowing. All seeds were treated with fludioxonil and metalaxil-m (Celest XL®, Syngenta Crop Protection S.p.A., Milan, Italy). All the plots received the same amount of nutrients: before sowing 100 kg ha^{-1} of K_2O (as potassium chloride) were applied, whereas during sowing 39 kg ha^{-1} of N and 100 kg ha^{-1} of P_2O_5 (as diammonium phosphate). At GS 16, 261 kg ha^{-1} of N (as urea) were applied as side-dressing fertilization, during the 25th and the 16th of May respectively in 2013 and 2014.

Irrigation was conducted using the pivot method, providing each time 50 mm of water, in order to avoid any drought stress until the end of the dough stage (GS 87). Irrigation was performed twice in 2013, whereas it was only applied once in 2014, due to abundant rainfall in the summer. Weed control was conducted at pre-emergence with mesotrione (0.15 kg AI ha^{-1}) S-metolachlor (1.25 kg AI ha^{-1}) and terbuthylazine (0.75 kg AI ha^{-1}) (Lumax®, Syngenta Crop Protection S.p.A., Milan, Italy). All the plots were sprayed at GS 75 in both trials with pyrethroid lambda-cyhalothrin insecticide (Karate® Zeon, Syngenta Crop Protection S.p.A., Milan, Italy) at 0.019 kg AI ha^{-1}. The spraying application was performed on July 19th and 22nd, respectively, for the 2013 and 2014 growing seasons. This allowed any possible damage to the stalks and ears, due to the larva activity of the European Corn Borer (ECB) (*Ostrinia nubilalis* Hübner), to be restrained.

2.1.2 Experiment 2

The standard planting density (StD) was compared against the innovative high planting density (HiD) for several production situations (PS) (Aubertot and Robin, 2013) from the year 2011 to 2014. The StD involved the ordinary planting pattern and density that are commonly adopted in the maize temperate growing areas of Europe and North America. This consists of an average seeding rate of 7.5 plants m^{-2}, planted in rows 0.75 m apart, with an average intra-row spacing of 0.18 m. The HiD system had the purpose of maximizing grain yield by increasing the seeding rate to 10.5 plants m^{-2}. In order to achieve this density, and at the same time to keep plants as equidistant as possible, the distance between rows was reduced to 0.5 m. The average gap between one plant and another was set to 0.19 m within each row. On both systems, planting was conducted by a mechanical sowing machine with adjustable row width. All seed were treated with fludioxonil and metalaxil-m (Celest XL®, Syngenta Crop Protection S.p.A., Milan, Italy).

Overall, the comparisons between the StD and HiD systems were made considering 34 PS, according to a completely randomized block design with 4 replicates. All the plots were 10 m long and 8 or 12 rows wide, for the StD and HiD systems, respectively. All the measurements were conducted on the two middle rows. The list of the compared PS and their main geographical and agronomical information is reported on Table 1.

A 0.3 m deep ploughing and an insecticide treatment were applied at GS 75 in all the trial fields in order to restrain the ECB activity. All the hybrids in the comparison belonged to a full maturity class (FAO 500 or 600) and they are representative of the varieties more cultivated in the considered growing area. As described in Table 1, different irrigation systems were adopted, according to the typical farm management system in force in the area, each providing enough water to maintain the water-holding capacity between 33 and 200 kPa. Other agronomical practices that have not been reported, such as fertilization and weed control, were conducted according to the ordinary agronomic techniques of the cultivation area and were the same for both density theses.

2.2 Field and harvest assessments

2.2.1 Experiment 1

The following assessments have been made on the two middle rows from each plot during the milk stage (GS 75), which occurred on August 12th and July 20th respectively for 2013 and 2014 growing seasons. The ear leaf greenness was measured by means of a chlorophyll meter, Hydro N-Tester® data logger (HNT) (Hydro-Agri, now Yara, Yara Italia S.p.a., Milan, Italy). Thirty randomly selected ear leaves were measured for each plot in order to record the average HNT value.

In order to measure the stalk area, t~~T~~he maximum (D) and minimum diameter (d) were measured on the first internode at the milk stage (GS 75) by means of a digital gauge. This assessment was conducted on 15 randomly selected plants per plot. The stalk area was then calculated using the following formula:

$$(1)\ Stalk\ area\ (cm^2) = \left(\frac{D\ (mm)}{2} * \frac{d\ (mm)}{2} * \pi\right)/100$$

The ear insertion height from the ground was measured at the milk stage (GS 75) on 15 plants randomly selected per plot.

Incidence of lodged plants was recorded for each plot before harvesting.

At the end of maturity, the grain yields were obtained by harvesting the entire length of the 2 middle rows in the 0.75 m SIS plots and the 3 middle rows in the NIS plots using a Walter Wintersteiger cereal plot combine-harvester. The grain yield results were adjusted to a 14% moisture content. This operation was carried out on October 15th and October 6th for the 2013 and 2014 growing seasons, respectively.

Before the mechanical harvest, ears were collected by hand and counted from each plot, considering a surface of 4.5 m^2, in order to record the number of lodged plants, to calculate the density per square meter of the fully developed ears (ESQ) and to conduct further assessments regarding the morphology of the ears and kernels.

The cob and tip back length was measured on 15 randomly selected ears. Moreover, the number of kernel rows (KR) and the number of kernels per row (KPR) were also counted on 7 of these randomly selected ears. The theoretical amount of kernels per square meter (KSQ) was then calculated by multiplying the average number of kernels per ear (KR * KPR) by the ESQ.

The collected ears were shelled using an electric sheller. The kernels from each plot were mixed thoroughly to obtain a random distribution; a 1 kg sub-sample was then taken and dried at 60°C for 72 hours, in order to reduce the kernel moisture content to 10%.

The grain moisture and the test weight (TW) were determined on wet and dry kernels, respectively, by means of a Dickey-John GAC2000 grain analysis meter (Dickey-John Corp. Auburn, IL, USA) according to the supplied programme. Two hundred dry kernels were randomly collected and weighed to assess the thousand kernel weight (TKW). The kernel volume (KV) was measured by recording the rise in the known alcohol volume after inserting 100 kernels into a graduate cylinder. Two analytical replicates were carried out for all these assessments. The average kernel length (KL) and kernel depth (KD) was measured on 30 kernels randomly selected from each sample using a digital gauge.

2.2.2 Experiment 2

At the end of maturity, ears were collected manually from an area of 4.5 m^2 in each plot, weighed, counted and then shelled. The following assessments were conducted on the kernels, according to the same procedure as experiment 1: grain moisture, TW, TKW, KR, KPR and KSQ. The ear weight (including the cob and grains) was calculated as the total weight of the ears divided by the number of harvested ears.

2.3 Statistical analysis

An analysis of variance (ANOVA) was utilized to compare the effect of the planting density of experiment 1 on the recorded parameters, using a split-plot design, in which the inter-row space and hybrid were considered as the main factors, whereas the four planting densities were considered as a sub-plot factor.

The normal distribution and homogeneity of variances were verified by performing the Kolmogorov–Smirnov normality test and the Levene test, respectively.

As far as grain yield and ESQ are concerned, ANOVA was conducted considering the combination of planting density, the inter-row spacing (8 theses) and the hybrid as independent variables. As far as KPR is concerned, the ANOVA test was conducted for each hybrid, considering the combination of planting density and the inter-row spacing as independent variables. The year was considered as a random variable in all the tests.

Multiple comparison tests were performed, according to the Ryan-Einot-Gabriel-Welsch F (REGW-F) test, on the planting density treatment means. SPSS Version 21.0 for Windows statistical package was used for the ANOVA statistical analysis.

The relative ratio (RR= HiD/StD) between the HiD agronomic system and StD was calculated for each measured parameter for all the PS considered in experiment n° 2. The average RR and the confidence interval were calculated at significance levels of 99.9% ($P<0.001$), 99% ($P<0.01$) and 95% ($P<0.05$).

3. Results

3.1 Experiment 1

The two years in which the experiment was carried out showed different meteorological trends throughout each growing season (Table 2). 2013 was characterized by abundant rainfall in April and May, which delayed planting operations. The first stage vegetative growth of the crop was also slowed down. On the other hand, the following year had a drier and warmer spring, which allowed precocious planting, although the summer was rainy and mild, which prevented heat and drought stress conditions.

Table 3 shows the effects of the hybrid, inter-row spacing and planting density on the morphological assessments performed on the plants, ears and kernels, ESQ and grain yield and moisture. The two compared hybrids differed significantly from each other for all the parameters measured, except for the ESQ and TW.

The average cob and tipback length of the fixed hybrid resulted to be longer than the flex one, that is, +6.7 and +9.9%, respectively. The KPR was also higher, while the KSQ was significantly lower (-13.4%), and this was mainly due to a reduced KR (15.7 rows versus 19.4). The KV was also higher (+5.4%) as was the TKW (+4.7%) and KL (+3.5%), whereas the KD was lower (-6.6%).

Of all the parameters related to plant architecture, the ones that were influenced the most by inter-row spacing were the stalk area and ear height. The plants in the narrow row system (NIS) had a thicker stalk (4.1 versus 3.7 cm^2, +8.9%) but also a slightly higher (+5 cm) ear height. The planting density significantly influenced the stalk development and ear height, but also the leaf greenness during the ripening stage, the latter being an indicator of the nutritional status of the crop. The reduction in the stalk area from the lowest population density (D1: 7.5 plants m^{-2}) to the highest (D4: 12 plants m^{-2}) was of 20%, whereas it was -5.2% for leaf greenness, which was expressed as an NHT value. Even though the increased planting population led to thinner plants, which were also characterized by a taller ear height, none of the compared treatments displayed a lodging propensity, and plant lodging was lower than 2% for both of the considered growing seasons. The interactions between inter-row space and planting density for these plant morphological parameters were never significant.

As far as the ears are concerned, the cobs belonging to the NIS plots resulted to be on average shorter than those from the 0.75 m wide rows. The KR value remained stable for the two inter-row spacings and the planting densities, while the KPR decreased consistently as the density was increased from 9 (D2) to 10.5 plants m^{-2} (D3). A significant interaction between hybrid and inter-row spacing was reported. A diminishing trend of KPR was recorded for both types of hybrid as a consequence of the increasing plant density. However, the effect was more consistent and significant on the flex hybrid (figure 1). The lowest KPR (34 kernels row^{-1}) was in fact observed on this hybrid on ears belonging to the plots planted at D4 with 0.5 m wide inter-rows (NIS). Nevertheless, this value did not differ from the D3 and D4 theses for the plants with the standard inter-row spacing (SIS). The highest KPR was observed for the lowest planting densities for both inter-row spacings. The difference in KPR in the flex hybrid for the NIS, between the two opposite planting densities (D1 vs D4) was higher than that measured for the standard planting density (6 kernels vs 4).

As expected, the average number of ears harvested per unit area (ESQ) significantly increased as the growing density increased. This parameter resulted to be 8.9% higher in the NIS than in the SIS, which also explains the 9.9% KSQ difference between the compared inter-row spacing systems. Figure 2 reports the ESQ values for the different planting densities for the NIS and the SIS. Only in the D1 thesis were the ESQ values from both inter-row planting patterns close to the target planting density of 7.5 plants m^{-2} (7.7 ears m^{-2} for the NIS and 7.2 ears m^{-2} for the SIS). For the higher plant densities, the number of ears harvested per unit area was on average lower than the number of plants per unit area, foreseen in the experimental protocol. The gap between the harvested ears (ESQ) and the number of plants per unit area resulted to gradually rise as the planting density was increased. Furthermore, this trend was clearly more consistent for the SIS than for the NIS: the average ESQ in the D4 thesis (12 plants m^{-2}) for the NIS was 11.3 ears m^{-2} and it was significantly different from the D4 thesis for the SIS, which resulted in an average of 10 ears m^{-2}. The same trend was observed for the D3 thesis: an average of 10.2 ears m^{-2} were harvested for the NIS, whereas only 9.1 ears m^{-2} were harvested for the SIS.

A significant interaction was observed between the inter-row and planting density on grain yield (figure 3). In the SIS, the grain yield did not increase because of the higher planting density. On the other hand, the NIS showed a significant yield benefit when the plant population was increased from 7.5 to 10.5 plants m^{-2} (+7.4%). The highest yield peak was obtained for 12 plants m^{-2} (D4) in the NIS. However, for this inter-row space systems this plant density did not differ significantly in grain yield from 10.5 plants m^{-2} (D3 thesis).

No significant differences were observed for grain yield between NIS and SIS at the lower plant density (D1 and D2). However, the NIS resulted in a significantly greater grain yield (+10.9%) than the SIS for the higher plant densities (D3 and D4).

The different inter-row spacing did not influence any of the kernel parameters, that is, moisture, TW, TKW, KL, KD or KV. On the other hand, the more intense plant population negatively influenced the length, volume and weight of the kernels. The TKW in fact diminished by 7.1% for between 7.5 and 12 plants m^{-2}, whereas the KV diminished by 5.7%. The interactions between the inter-row spacing and planting density were never significant for the kernel parameters.

3.2 Experiment 2

The results of the application of the innovative high plant density system (HiD), compared to the standard one (StD), in the 34 production situations (PS), are reported in Table 4. The average yield increase obtained by switching from StD to the HiD was of 1.8 t ha^{-1} (+11%, P<0.001). The grain moisture, TW and KR were not affected by the two different seeding rates. Conversely, the TKW decreased by 5.7% from the StD to the HiD. The average ear weight dropped by 18% after the adoption of the innovative system. The same trend, but less pronounced (-9%) was seen for the KPR value. However, because of the higher number of ears per square meter, the total amount of kernels per square meter (KSQ) increased (+23%).

Figure 4 represents the percentage of grain yield variation obtained after adopting the HiD system, sorted from the lowest to the highest in all 32 PS taken into account. Only 6.2% of the cases (2 PS out of 32) showed a negative effect, with an average yield decrease of 6.8%, while one PS showed no variations. The remaining cases (90%) instead showed a yield increase of between 1.3% and 22%.

4. Discussion

The study has proved that the morphological adaptation of plants, ears and particularly of grain yield response is clearly influenced by the equidistance space between plants, and therefore by plant density and inter-row spacing.

Data collected during an extensive comparison of production situations (Table 1) have confirmed that when the seeding rate increases up to 10.5 plant m^{-2}, the ear and kernel development is modified, with a lower single plant yield potential. However, because of the higher number of kernels harvested per unit area when higher density systems were adopted, the final grain yield was clearly enhanced.

The KPR, unlike the KR, has been shown (Table 3) to be negatively affected by an increase in planting population, especially for the flex ear hybrid. The young kernel abortion immediately after fertilization, is due to a lower supply of carbon and nitrogen to the ear (Liu et al., 2015). In high density conditions, in fact, the crop has to face a more challenging and constraining situation, which is mainly caused by intra-specific competition. This starts to occur above all at the end of the stem elongation and grain development stage (GS 38-70), which is a key period in determining the number of KPR. KR is instead defined at an earlier stage, usually from the fifth to the eight leaf unfolded stage (GS 15-18) (Dardy and Lauer, 2006). During this period, the crop is not yet in an intra-specific competitive environment caused by the higher planting density. Plants in fact are still too small to cause a stressful condition to each other. In the present experiment, since the KPR decreased while the KR remained the same, the total number of kernels produced by a single plant was reduced because of the higher plant population, as already reported by Echarte et al. (2000) and Zhang et al. (2014) for an increasing planting population with a steady inter-row spacing of 0.70 m.

As far as the kernel dimension is concerned, the increase in plant density caused a reduction in kernels, which was related to the lower kernel length and volume (Table 3), whereas no effects were observed for the variations in row width. This also explains why the smaller kernels caused a lower TKW, which, among others, was also seen by Sangoi et al. (2002). On the other hand, the TW remained stable for the different compared plant densities in both experiments 1 and 2 (Table 3 and 4). A possible explanation could be that, although the single grain was not so heavy, smaller kernels were able to better fill the fixed volume thus reducing the empty spaces more and compensating for the lower single kernels weight.

All the previously discussed aspects confirm that compared to the standard plant density for full maturity hybrids a higher planting population, which implies fewer, smaller and lighter kernels per ear, lowers the yield potential of the single maize plant (Li et al., 2015). As can clearly be seen from the multiple comparison of experiment 2 (Table 4), the average ear weight decreased by 18% for the high plant density system. However, the key to the yield enhancement obtained from the application of a high plant population in modern hybrids, is the higher number of kernels harvested per unit area (KSQ), which was +26% and +23% for experiment n° 1 and 2, respectively, comparing 7.5 and 10.5 plants ha^{-1}. For highly competitive conditions, which are typical of high plant populations, the final grain yield is linked more to the number of harvested kernels than to the kernel weight itself (Sangoi et al., 2002). In the environmental conditions in which experiment 1 was conducted, a significant yield increase for a higher plant population (10.5 plant m^{-2}) was only observed in NIS (0.5 m inter-row distance), and not in the SIS (0.75 m inter-row distance) (Figure 3). The main advantage of the NIS was that a higher number of ears per area unit (ESQ) was harvested than for SIS (Figure 2). In fact, the plants grown with a high plant density in the SIS had a higher incidence of barrenness than those grown in the NIS. This is the consequence of the lower equidistance space that was adopted in the SIS: by maintaining the 0.75 m inter-row width and moving from 7.5 plants m^{-2} to 12 plants m^{-2}, the average distance of two contiguous plants on the same row was reduced from 0.18 m to 0.11 m. With a high increase in the plant population for a large inter-row spacing, the risk of having higher irregularities of the plant distance in the row is greater and consequently the percentage of dominated plants, which are often infertile is also higher (Lauer and Rankin, 2004). However, the NIS system allows a more even distance between intra-row plants and thus alleviates intercrop competition, therefore only in this condition, a reduced plant spacing, yet more equidistant, allowed a significant yield increase. The better response of NIS has confirmed what was observed in previous studies on silage maize (Cox and Cherney, 2001). Farnham (2001) also reported a better yield increase when adopting higher planting populations for an even narrower (0.38 m) inter-row space, although the average grain yield was higher for a 0.76 m wide one. In other studies in which the highest plant density tested was 10 plants m^{-2} (Nielsen, 1988; Porter et al., 1997; Widdicombe and Thelen, 2002), it was reported that row width was inversely correlated to the grain yield and that an interaction between plant density and row width was observed. The use of light, with a better interception of the solar radiation, is more efficient on narrow rows, and contributes by maximizing the final yield (Onyango, 2009). This type of planting leads to a better shading of the soil surface during the early part of the season, and limits water evaporation from the bare soil, as reported by Bullock et al. (1998) for soybean. Moreover, a more shaded inter-row space does not offer a suitable environment for weeds. The NIS in fact could reduce competition with weeds through early canopy closure (Fanadzo et al., 2010). This planting pattern, although resulting in a more enhanced yield when a high planting density is adopted, needs to be supported with appropriate field equipment. The tractor wheels and track base width have to be dimensioned correctly as well as the combine harvester header and harrow squadrons, in order to prevent any driving over plants. Furthermore, it is expected that the inter-row tillage in a NIS would be shallower, thus limiting soil ventilation, water penetration and mechanic weed control. However, no further difficulties appeared for the irrigation process, in either the furrow inter-row method, or in the over canopy ones (sprinkling/pivot systems).

The hybrids evaluated in experiment 1, after the application of the higher planting population, showed different behavior, in terms of ear development, but also in terms of plant architecture. However, their response to the higher plant population was similar in terms of yield increase, since no interaction was seen between these factors.

The highest planting density sown at 12 plants m^{-2} was not sufficient to determine a yield breakdown of either the inter-row spacing or the hybrids. However, this planting density treatment did not significantly differ from when the plants were sown at a rate of 10.5 plants m^{-2} (Figure 3), thus confirming that, for the agronomical conditions that were tested, the best crop yield potential was obtained for 10.5 plants m^{-2}. This work has shown that, in growing areas which are suitable for maize cultivation, under irrigated and fertile conditions, an increase in planting density from 7.5 to 10.5 plants m^{-2} with the present full season hybrids represents a real opportunity for farmers to boost the crop yield potential. This agronomical strategy, albeit very promising, needs to be considered carefully in the different production situations, since the risk of losses related to greater stress can increase. As far as plant stand ability is concerned, the stalk section was shown to be negatively affected by the higher planting population (Table 3), whereas the ear height had an opposite trend. Plants sown more densely, receive a different quality of light, characterized by enriched far red and impoverished red radiation (Rajcan and Swanton, 2001). This can in fact stimulate apical dominance, and induce plants to grow taller, with a thinner stalk that is more disposed to lodging or breaking (Sangoi et al., 2002; Stanger and

Lauer, 2007; Widdicombe and Thelen, 2002). Even though ear insertion tended to be higher for the NIS, the stalk area was also larger, and hence more tolerant to plant lodging. Moreover, it has also been reported for maize, that some chemical compounds, known as plant growth regulators (PGRs), can modify plant growth by reducing height and thus lodging risks when a high planting density is applied or in other conditions (Shekoofa and Emam, 2008; Zhang et al., 2014). In the fields where the trials were carried out, lodging was not observed to be connected to any treatment. Similar results have also been recorded in experiments conducted on lodging tolerant hybrids in Brazil (Sangoi et al., 2002) on densities of 10 plants m^{-2}.

The decrease in leaf greenness, due to the increase in plant population (Table 3), could be interpreted as a response of the crop to the lower nutrient supply available for each single plant, especially in terms of N uptake (Wood et al., 1992). This aspect has also been confirmed by the fact that no differences were observed between the two inter-row spacings. In fact, in the considered greater planting population conditions, the same amount of nutrients was diluted over a higher number of plants. This underlines that a high planting density is less suitable for fields characterized by lower soil fertility, or when the supply of nutrients through the fertilization, and N in particular, is not adequate for the higher yield level. The nutrient balance in a high density crop system is certainly different from a standard one, since a higher nutrient uptake is expected (Rao et al., 2014). Moreover, a high density crop could be more sensitive to drought stress: water consumption is greater in high stands, since more water evapotraspirates through the plants, although less water evaporates from the bare ground. This enhances the crop water use efficiency, but conversely increases crop requirements. Thus, in no irrigated or in less water availability conditions, lower plant populations are preferred (Karlen and Camp, 1985).

In addition to the data reported in this work, it is also necessary to consider the impact on kernel quality in order to carefully evaluate the applicability of high population planting of maize for grain. The lower nutrient and water availability for each plant in the high density system could affect not only the single plant yield potential, but also other kernel quality parameters (Oikeh et al., 1998), such as grain composition and kernel hardness. Moreover, the higher stress condition could negatively affect the sanity of the grain yield. In a previous work (Blandino et al., 2008) a higher planting population in StD (8.2 compared to 6.5 plants m^{-2}) led to an increased fungal ear rot severity and mycotoxin contamination. However, at the moment, no qualitative data are available for the HiD condition, pertaining to a more balanced distance between the plants; therefore further evaluations focused more on kernel quality are needed. This will lead to a better understanding of whether the adoption of a high planting system on a narrow inter-row space could enhance farmer competitiveness on the market by increasing grain yield, and at the same time provide the high quality standards required for the supply chain.

5. Conclusion

This work has proved that, for the cultivation of medium-late maturing hybrids in temperate areas and irrigated conditions, a high planting density of up to 10.5 plants m^{-2} can lead to a significant yield increase, only when it is combined with narrow inter-row spacing, since it guarantees a more plant equidistance. The high density condition increases plant stresses, and modify plant morphology and development to the detriment of the single plant yield. However, the lower yield per plant is fully compensated by the higher plant population.

6. Acknowledgements

The authors would like to thank Francesco Amato, Giovanni Berrino, Matteo Calcagno, Alessandra Gazzola, Federico Marinaccio, Andrea Pilati, Giacomo Sala, Valentina Scarpino and Stefania Stura for their expert technical assistance. The funds for this research were provided by KWS Italia S.P.A.

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TABLES

Table 1. List of production situations (PS) in which experiment 2 was carried out. Year, location, growing degree days (GDD) from April to October, soil texture, hybrid, and planting and harvest dates are reported.

# PS	Year	Location[a]	GDD April-October (°C d^{-1})	Soil texture	Hybrid	Planting Date	Harvest Date	Irrigation method
1	2011	Carignano	2089	Loam	SY NX7234	26-Mar	14-Sep	Furrow
2	2011	Carignano	2089	Loam	SY NX7234	14-Apr	21-Sep	Furrow
3	2011	Carmagnola	2072	Silt loam	P1543	8-Apr	19-Sep	Sprinkling
4	2011	Verolengo	2053	Sandy loam	SY NX7234	4-Apr	8-Sep	Furrow
5	2012	Buriasco	2014	Silt loam	DKC 6815	26-Mar	3-Oct	Pivot
6	2012	Buriasco	2014	Silt loam	SY NX7234	26-Mar	3-Oct	Pivot
7	2012	Macello	2023	Silt loam	SY NX7234	26-Mar	3-Oct	Pivot
8	2012	Buriasco	2014	Silt loam	KWS Korimbos	26-Mar	3-Oct	Pivot
9	2012	Macello	2023	Silt loam	KWS Korimbos	26-Mar	3-Oct	Pivot
10	2012	Buriasco	2014	Silt loam	P1758	26-Mar	3-Oct	Pivot
11	2012	Buriasco	2014	Silt loam	SY Radioso	26-Mar	3-Oct	Pivot
12	2012	Carignano	1995	Loam	SY NX7234	27-Mar	8-Oct	Furrow
13	2012	Carignano	1995	Loam	P1547	27-Mar	8-Oct	Furrow
14	2012	Carignano	1995	Loam	SY Radioso	27-Mar	8-Oct	Furrow
15	2012	Carmagnola	2002	Silt loam	P1547	30-Mar	26-Sep	Sprinkling
16	2012	Verolengo	1928	Sandy loam	SY NX7234	23-Mar	24-Sep	Furrow
17	2012	Verolengo	1928	Sandy loam	SY Radioso	23-Mar	24-Sep	Furrow
18	2013	Buriasco	1911	Silt loam	SY NX7234	17-Apr	15-Oct	Pivot
19	2013	Macello	1918	Silt loam	SY NX7234	17-Apr	15-Oct	Pivot
20	2013	Buriasco	1911	Silt loam	KWS Korimbos	17-Apr	15-Oct	Pivot
21	2013	Macello	1918	Silt loam	KWS Korimbos	17-Apr	15-Oct	Pivot
22	2013	Carignano	1875	Loam	SY Radioso	13-May	28-Oct	Furrow
23	2013	Carmagnola	1879	Silt loam	P1547	9-May	4-Nov	Sprinkling
24	2013	Vigone	1911	Silt loam	P1547	13-May	22-Oct	Furrow
25	2013	Vigone	1911	Silt loam	PR34G44	13-May	22-Oct	Furrow
26	2014	Buriasco	1842	Silt loam	DKC6815	17-Mar	2-Oct	Furrow
27	2014	Buriasco	1842	Silt loam	SY NX7234	17-Mar	6-Oct	Furrow
28	2014	Buriasco	1842	Silt loam	KWS Korimbos	17-Mar	6-Oct	Furrow
29	2014	Buriasco	1842	Silt loam	P1547	17-Mar	2-Oct	Furrow
30	2014	Carmagnola	1979	Silt loam	P1547	21-Mar	6-Oct	Sprinkling
31	2014	Chivasso	1844	Sandy loam	SY NX7234	4-Apr	29-Sep	Furrow
32	2014	Chivasso	1844	Sandy loam	KWS Korimbos	4-Apr	29-Sep	Furrow

[a] GPS coordinates for each location: Carignano N44°52'48'' E7°37'42''; Carmagnola N44°53'11'' E7°41'15''; Verolengo N45°13'47'' E7°56'41''; Buriasco N44°51'48'' E7°26'20''; Macello N44°51’21’’ E7°25’44’’; Vigone N44°50'19'' E7°28'55''; Chivasso N45°12'42'' E7°55'41''.

Table 2. Monthly total rainfall, rainy days, average temperature and growing degree days (GDD) from April to October in Buriasco in the 2013 and 2014 growing seasons.

Year	**2013**				**2014**			
Month	**Rainfall (mm)**	**Rainy days (d)**	**Average T (°C)**	**GDD (°C d^{-1})**	**Rainfall (mm)**	**Rainy days (d)**	**Average T (°C)**	**GDD (°C d^{-1})**
April	206	17	12.6	124	71	12	14.3	163
May	231	21	14.8	182	91	13	16.4	210
June	42	9	21.0	326	102	12	20.7	324
July	93	8	24.2	428	153	18	20.6	337
August	25	9	23.1	405	50	9	20.6	345
September	11	7	19.3	297	132	11	18.4	277
October	85	15	13.5	148	24	9	14.6	187
April-October	694	86	18.4	1911	623	84	17.9	1842

Table 3. Effect of hybrid, inter-row spacing and planting density on the stalk area, ear height, leaf greenness (N-tester), cob length, tip back length, kernel rows (KR), kernels per row (KPR), kernels per square meter (KSQ), ears per square meter (ESQ), grain yield, grain moisture, test weight (TW), thousand kernel weight (TKW), kernel length (KL), kernel depth (KD) and kernel volume (KV).

Factor	**Source of Variation**	**Stalk area (cm^2)**	**Ear height (cm)**	**Leaf greenees (NHT value)**	**Cob lenght (cm)**	**Tip back (cm)**	**KR (n)**	**KPR (n)**	**KSQ ($n\ m^{-2}$)**	**ESQ ($n\ m^{-2}$)**	**Grain Yield ($t\ ha^{-1}$)**	**Grain Moisture (%)**	**TW ($kg\ hL^{-1}$)**	**TKW (g)**	**KL (mm)**	**KD (mm)**	**KV (mm^3)**
Hybrid[a] (A)	Fix	3.6 b	133 b	879 a	20.1 a	2.02 a	15.7 b	40.7 a	5692 b	9.0	17.0 a	27.7 a	77.1	364 a	13.34 a	4.27 b	297 a
	Flex	4.2 a	159 a	806 b	18.7 b	1.82 b	19.4 a	36.7 b	6453 a	9.2	15.8 b	27.2 b	76.9	346 b	12.87 b	4.55 a	281 b
	P	***	***	***	***	***	***	***	***	NS[b]	***	**	NS	***	***	***	***
	SEM[c]	*0.54*	*7.95*	*29.7*	*0.90*	*0.33*	*1.28*	*3.04*	*924*	*1.10*	*1.39*	*1.22*	*1.16*	*18.0*	*18.0*	*0.12*	*16.8*
Inter-row spacing[d] (B)	Narrow (NIS)	4.1	148	844	19.2	1.95	17.6	38.8	6385 a	9.5 a	17.0 a	27.4	77.1	353	13.17	4.39	289
	Standard (SIS)	3.7	143	840	19.7	1.89	17.4	38.7	5755 b	8.7 b	15.8 b	27.5	77.0	357	13.06	4.42	290
	P	***	***	NS	**	NS	NS	NS	***	***	***	NS	NS	NS	NS	NS	NS
	SEM	*0.54*	*7.95*	*29.7*	*0.90*	*0.33*	*1.28*	*3.04*	*924*	*1.10*	*1.39*	*1.22*	*1.16*	*18.0*	*18.0*	*0.12*	*16.8*
Planting Density[e] (C)	D1	4.4 a	143 b	867 a	20.7 a	1.73 c	17.7	41.0 a	5380 c	7.5 d	15.8 b	27.1	77.0	369 a	13.35 a	4.44	298 a
	D2	4.0 b	145 ab	850 b	19.9 b	1.89 bc	17.5	39.7 a	5770 c	8.4 c	16.2 ab	27.5	76.9	360 a	13.21 a	4.42	293 ab
	D3	3.6 c	147 ab	831 c	18.8 c	1.95 ab	17.5	37.6 b	6279 b	9.6 b	16.7 a	27.7	77.1	350 b	12.94 b	4.38	286 bc
	D4	3.5 c	148 a	821 c	18.4 c	2.09 a	17.4	36.8 b	6758 a	10.7 a	16.9 a	27.5	77.1	343 b	12.97 b	4.39	281 c
	P	***	*	***	***	***	NS	***	***	***	**	NS	NS	***	***	NS	**
	SEM	*0.76*	*11.24*	*42.0*	*1.27*	*0.47*	*1.81*	*4.29*	*1307*	*1.56*	*1.96*	*1.72*	*1.64*	*25.4*	*25.4*	*0.17*	*23.7*
A x B	P	NS	***	*	**	NS	NS	**	NS	NS	NS	NS	NS	*	***	*	**
A x C	P	**	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
B x C	P	NS	NS	NS	NS	NS	NS	NS	NS	NS	*	NS	NS	NS	NS	NS	NS

Means followed by different letters are significantly different. The level of significance (P) is shown in table.

[a] The hybrid factor values are based on 48 replicates (2 years X 2 inter-row spacings X 4 planting densities X 3 repetitions). Fix hybrid: Syngenta NX7234, FAO maturity class 500; Flex hybrid: KWS Korimbos, FAO maturity class 500.

[b] NS, not significant (P(F)<0.05).

[c] SEM, standard error of means.

[d] The inter-row spacing values are based on 48 replicates (2 years X 2 hybrids X 4 planting densities X 3 repetitions). The narrow inter-row (NIS) is 0.5 m wide, while the standard inter-row (SIS) is 0.75 m wide.

[e] The planting density values are based on 24 replicates (2 years X 2 hybrids X 2 inter-row spacings X 3 repetitions). D1: 7.5 plants m^{-2}; D2: 9 plants m^{-2}; D3: 10.5 plants m^{-2}; D4: 12 plants m^{-2}.

Table 4. Comparison of the innovative planting density (HiD) and the standard density (StD) in different production situations[a] on grain yield, grain moisture, test weight (TW), thousand kernel weight (TKW), kernel rows (KR), kernels per row (KPR) and kernels per square meter (KSQ).

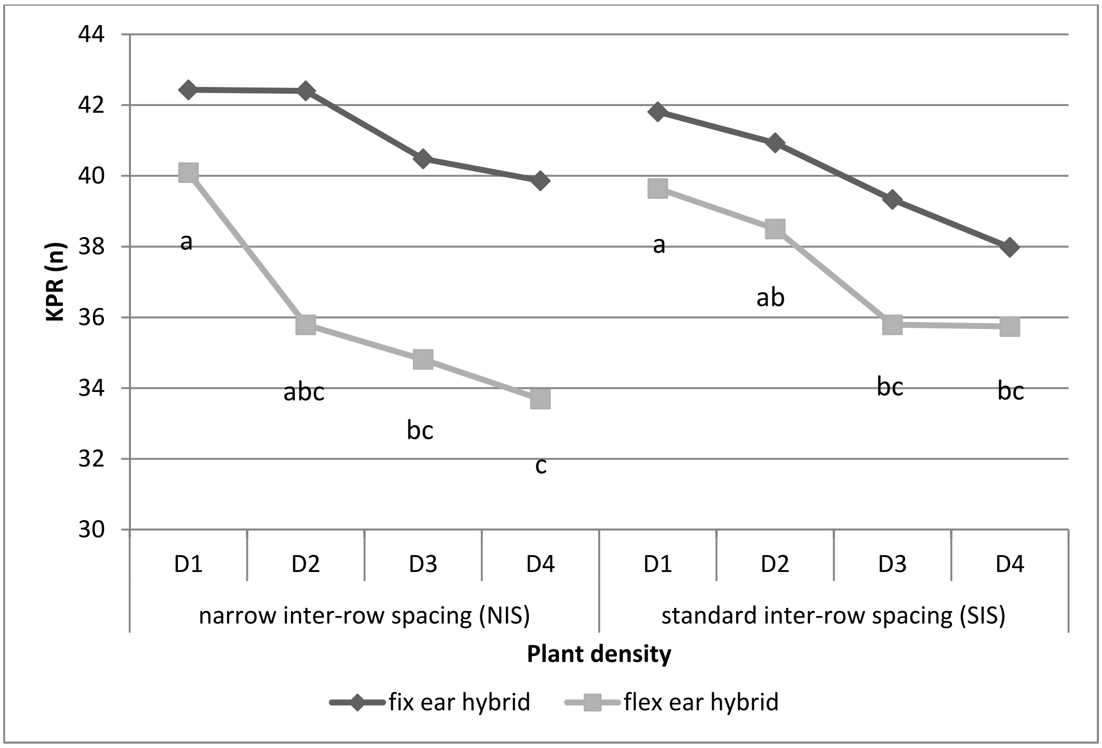
Parameter	Unit	Mean HiD	Mean StD	Mean RR	Significance[b]
Grain yield	t ha^{-1}	18.1	16.2	1.117	***
Grain moisture	%	25.4	25.0	1.013	NS[c]
TW	kg hL^{-1}	78.3	78.2	1.001	NS
TKW	g	372	394	0.942	***
Ear weight	g	260	317	0.824	***
KR	n	16.5	16.3	1.013	NS
KPR	n	36.4	40.2	0.904	***
KSQ	n m^{-2}	5775	4690	1.233	***

[a] The production situations refer to those reported in table 1.

[b] The reported values are mean for each treatment, relative ratio (RR = HiD/StD) and significance in term of interval of confidence.

[c] NS = not significant at P<0.05.

Figure 1. Effect of inter-row spacing and planting density on the number of kernels per row (KPR) developed on the fixed and flex ear hybrids.

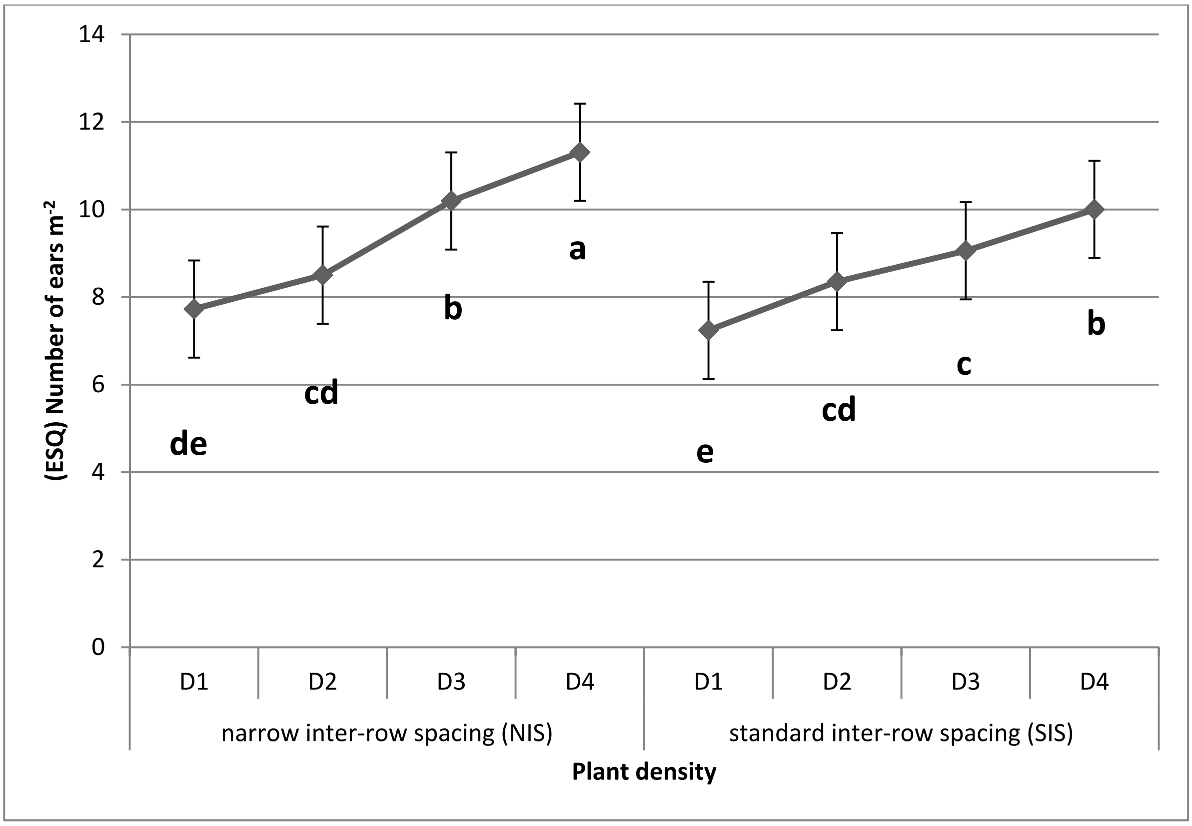


Means of the flex ear hybrid followed by different letters are significantly different for P<0.001 and a standard error of means (SEM) of 1.46. No significant differences were observed for the fixed ear hybrid (SEM=3.4).

The planting density X inter-row spacing values for each hybrid are based on 6 repetitions (2 years X 3 replicates). The NIS was 0.5 m wide, while the SIS was 0.75 m wide. Plant density: D1 7.5 plants m^{-2}; D2 9 plants m^{-2}; D3 10.5 plants m^{-2}; D4 12 plants m^{-2}.

Fix hybrid: Syngenta NX7234, FAO maturity class 500; Flex hybrid: KWS Korimbos, FAO maturity class 500.

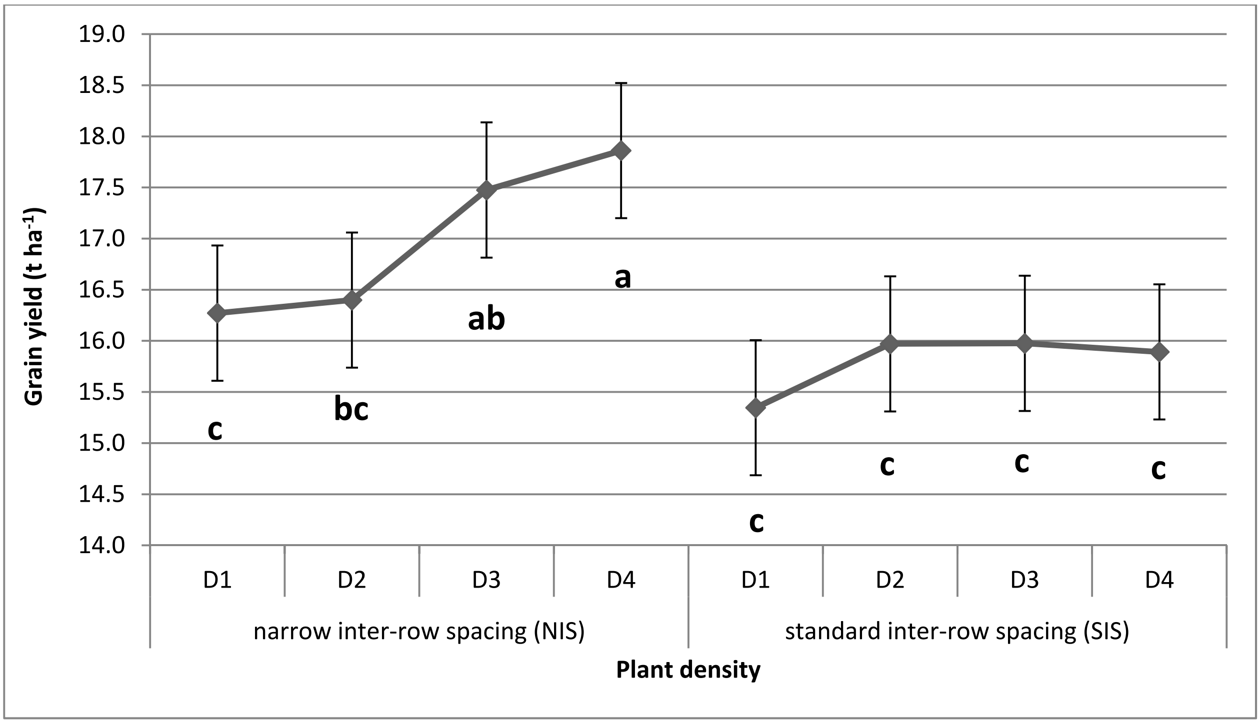
Figure 2. Effects of inter-row spacing and planting density on the number of ears per square meter (ESQ).



Mean values followed by different letters are significantly different ($P<0.01$).

Inter-row spacing X planting density values are based on 12 repetitions (2 years X 2 hybrids X 3 replicates). The narrow inter-row was 0.5 m wide, while the standard inter-row was 0.75 m wide. Plant density: D1 7.5 plants m^{-2}; D2 9 plants m^{-2}; D3 10.5 plants m^{-2}; D4 12 plants m^{-2}.

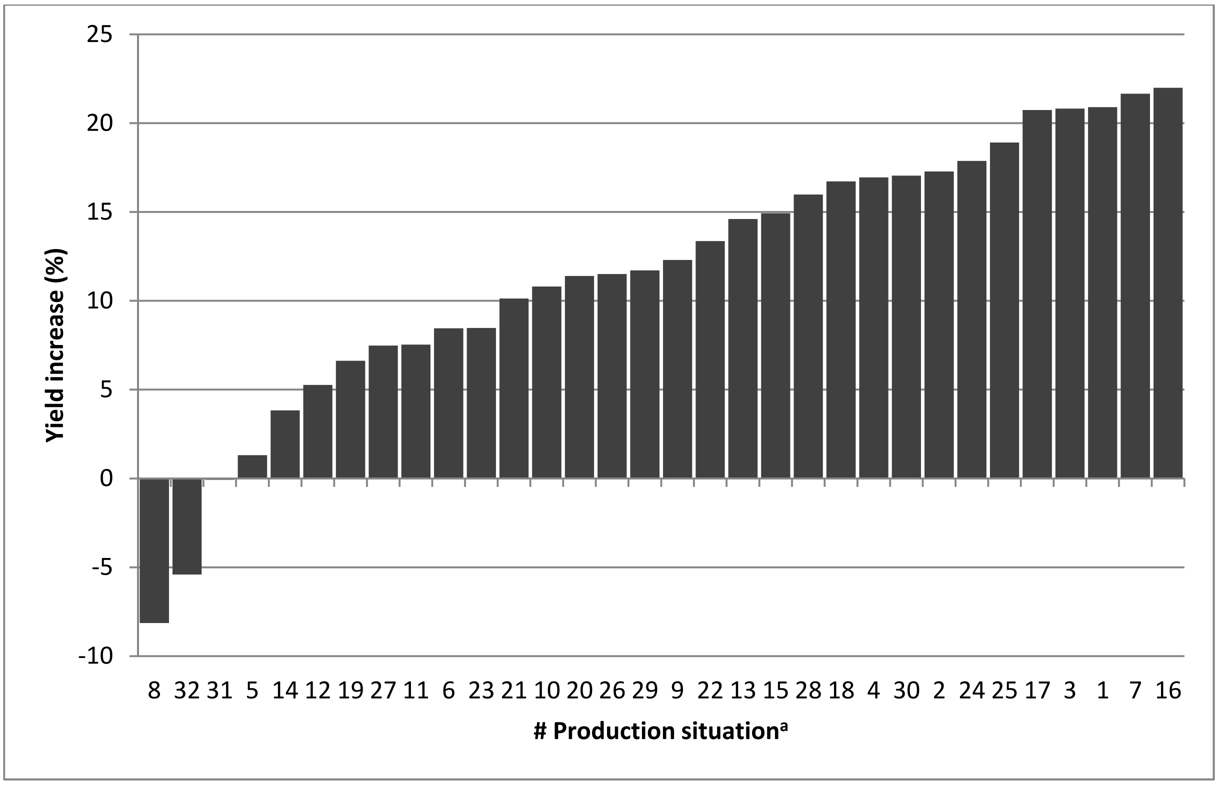
Figure 3. Effects of inter-row spacing and planting density on grain yield.



Mean values followed by different letters are significantly different ($P<0.01$).

Inter-row spacing X planting density values are based on 12 repetitions (2 years X 2 hybrids X 3 replicates). The narrow inter-row was 0.5 m wide, while the standard inter-row was 0.75 m wide. Plant density: D1 7.5 plants m^{-2}; D2 9 plants m^{-2}; D3 10.5 plants m^{-2}; D4 12 plants m^{-2}.

Figure 4. Percentage yield increase of the innovative planting density (HiD) compared to the standard density (StD) on the 32 compared production situations.



[a] The production situations refer to those reported in table 1.