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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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1 **Abstract**

2 Increasing the maize plant population has undergone a constant evolution over the years,
3 with the purpose of enhancing the crop yield. Field trials, in which 2 different experiments
4 were considered, have been conducted in NW Italy in order to analyze the yield benefit of
5 high plant density on recent hybrids, considering both intra-row and inter-row plant
6 spacings. The first experiment, which was carried out during the 2013 and 2014 season,
7 evaluated the effects of 4 growing plant densities (from 7.5-to 12 plants m⁻²) combined with
8 2 row widths (the traditional 0.75 m and a narrow inter-row spacing of 0.50 m) on plant
9 architecture-and grain yield. Two hybrids, with different ear developments (fixed and flex),
10 were taken into account. The higher plant density led to a decrease in the stalk area
11 (-20%), leaf greenness (-5.2%) and cob length (-10.8%). It also negatively affected the
12 kernel weight (-7.1%) and the number of kernels per row (-10%). The grain yield only
13 increased significantly, for both hybrids (+7.4%), if a density of 10.5 plants m⁻² was
14 reached when the inter-row spacing was reduced (0.50 m). In the second study, an
15 innovative system (narrow inter-row spacing combined with a plant population of about
16 10.5 plants m⁻²) was compared with a standard planting system (7.5 plants m⁻² sown on
17 0.75 m wide rows), considering 32 different production situations (PS) over 4 growing
18 seasons (2011-2014). This study has confirmed that even though the single plant yield
19 potential was reduced in terms of ear weight and thousand kernels weight by 18% and 6%
20 respectively for a high planting density, the final grain yield increased on 90% of the PS
21 with an average gain of 11.7%. This work has proved that, in the conditions in which the
22 experiments were conducted, a high planting density of up to 10.5 plants m⁻² can lead to a
23 significant yield increase, but only when it is combined with narrow inter-row spacing.
24 These conditions increase plant stresses, and modify plant morphology and development
25 to the detriment of the single plant yield. However, the lower yield per plant is fully

26 compensated by the higher plant population.

27

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

29

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

36 **1. Introduction**

37 Increasing the population density of plants is an agronomical practice that has
38 continuously been studied for maize crops. This crop technique has evolved and will
39 continue to evolve over the years and it is the agronomic management factor that has
40 changed the most over the past six decades (Tollenaar, 1992). After the introduction of the
41 first hybrids, farmers started to steadily increase the plant density, at an average rate of
42 0.3 plants m⁻² yr⁻¹. In the US Corn Belt of the 1930s, the mean population density was 3
43 plants m⁻², while it was 4 plants m⁻² in the 1960s and 6 plants m⁻² in the 1980s (Duvick,
44 2005). Nowadays, the average density in the USA, where maize cultivation is intense, is
45 around 8 plants m⁻² (Li et al., 2015), whereas in the EU, where the pedo-climatic
46 conditions are more heterogeneous across countries, it can vary from 6 to 8 plants m⁻² for
47 medium-late maturing hybrids in fertile growing areas. On both continents, in which this
48 crop is cultivated intensively, the most common inter-row spacing for the current planting
49 density is approximately 0.70 - 0.75 m in order to facilitate inter-row tillage (Sharratt and
50 McWilliams, 2005). The main purpose of increasing the plant density is to enhance maize
51 yield in terms of grain or biomass, thus making the crop system more efficient and
52 competitive per area unit. In the absence of biotic or abiotic stresses, grain yield is related
53 to the amount of solar radiation intercepted by the crop, and the use of a high density
54 population, with an earlier canopy closure, maximizes the leaf area index (Cox and
55 Cherney, 2001)

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

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

69 The shoot and seed density per area unit of modern maize hybrids is theoretically the
70 same, since these plants have lost their tillering ability (Duvick et al., 2004b), to the
71 advantage of a higher harvest index (HI) on the single plant. Thus, the only way that maize
72 plants can moderately compensate for planting density changes on the final yield is
73 through an adaptation of ear development. A flex ear hybrid adjusts its ear growth
74 according to the conditions that are encountered in the field, by modifying the number of
75 kernels per ear that reach full maturity. This feature allows, for example, to better
76 compensate for plant vacancies and hence preserve the yield if harsh field conditions
77 occur (Mascagni and Bell, 2004). On the other hand, a fixed ear development keeps the
78 total number of kernels per ear relatively steady, regardless of the environmental
79 conditions. Maize is one of the herbaceous species that offers the highest grain yield
80 potential, yet it is one of the most sensitive crops to variations in plant density (Vega et al.,
81 2001). Modern hybrids can generally withstand higher population densities, because they
82 can support stressful environmental conditions caused by a higher intra-specific
83 competition more easily, and this results in a lower incidence of barren plants and reduced
84 stem lodging (Tollenaar, 1989; Widdicombe and Thelen, 2002). In order to overcome the
85 problems of high density planting, these hybrids are characterized by more upright and

86 shorter leaves, and the synchrony between the differentiation of the female and male
87 florets is higher (Sangoi, 2000), thus limiting barrenness. However, in order to place the
88 crop in the best growing conditions possible, even for high plant populations, the planting
89 pattern has to be re-thought with a reduction in the inter-row spacing and a more balanced
90 equidistance. Plants spaced more uniformly in fact compete minimally for the main
91 growing factors. Among these factors, the one that is affected most is light, and this is
92 followed by nutrients and water (Li et al., 2015; Sharratt and McWilliams, 2005).
93 According to this line of reasoning, an increase in planting density, obtained by reducing
94 the within row plant spacing, could be a limiting factor. In fact, if plants are sown too close
95 to each other in the same row, their full yield potential could be restrained.

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

105 **2. Materials and methods**

106 **2.1 Experimental sites and agronomical management**

107 The study on the effects of different planting densities on the morphological development
108 of plants and ears and grain yield was performed over four growing seasons, from 2011 to
109 2014, and it involved two different experiments conducted in the same agricultural area in
110 the Piedmont region in North West of Italy, characterized by a humid subtropical climate
111 according to the Köppen climate classification (Peel, 2011).

112 **2.1.1 Experiment 1**

113 Field trials were conducted in the 2013 and 2014 growing seasons at Buriasco, in the
114 North West of Italy (44°51'53" N, 7°26'21" E), an area that is characterized by a deep and
115 fertile loamy soil (40% sand, 46% silt, 15% clay, Typic Hapludalfs, USDA classification)
116 and a medium-high content of organic matter (3.2%) with a balanced C/N ratio (11.0). The
117 N, P₂O₅ (available) and K₂O (exchangeable) soil content were respectively high (1.7 g
118 kg⁻¹), medium (23 ppm) and low (18 ppm). The soil pH was sub-acid (6.3) and the Cation
119 Exchange Capacity was medium (16.3 meq 100 g⁻¹).

120 The compared treatments were a factorial combination of:

- 121 - 2 inter-row spacings:
 - 122 ○ 0.75 m wide, standard inter-row spacing (SIS), representing the reference
 - 123 spacing for the maize crop system;
 - 124 ○ 0.5 m wide, narrow inter-row spacing (NIS)
- 125 - 4 planting densities:
 - 126 ○ D1: 7.5 plants m⁻² (reference planting density)
 - 127 ○ D2: 9 plants m⁻²
 - 128 ○ D3: 10.5 plants m⁻²

- 129 ○ D4: 12 plants m⁻²
- 130 - 2 hybrids characterized by a different ear development according to the
- 131 environmental conditions that occur during the growing season:
- 132 ○ Syngenta NX7234, FAO maturity class 500; 127 relative days to maturity,
- 133 characterized by a fixed ear development.
- 134 ○ KWS Korimbos, FAO maturity class 500; 125 relative days to maturity,
- 135 with a flex ear development.

136 The experiment was carried out according to a split plot design: the inter-row space and

137 hybrid were considered as the main factors, whereas the planting density was considered

138 as a sub-plot factor, with 3 randomized replicates. The whole field was planted at a

139 maximum planting density of 12 plants m⁻², and then each plot was thinned at the four leaf

140 stage (growth stage GS 14) (Lancashire et al., 1991) to adjust the plant population to the

141 desired levels. The plant density was then checked again by counting the plants from GS

142 65 to GS 75. The sub-plots for the 0.5 m inter-row spacing were 10 m. long and 12 rows

143 wide, and were 8 rows wide for the 0.75 m inter-spacing. The plot alleys, which were

144 orthogonal to the maize rows, were 1 m wide.

145 The previous crop was maize each year. Mechanical Pplanting was carried out on April

146 17th and March 17th, respectively, for 2013 and 2014, after an autumn 0.3 m deep

147 ploughing, followed by disk harrowing. All seeds were treated with fludioxonil and

148 metalaxil-m (Celest XL[®], Syngenta Crop Protection S.p.A., Milan, Italy). All the plots

149 received the same amount of nutrients: before sowing 100 kg ha⁻¹ of K₂O (as potassium

150 chloride) were applied, whereas during sowing 39 kg ha⁻¹ of N and 100 kg ha⁻¹ of P₂O₅ (as

151 diammonium phosphate). At GS 16, 261 kg ha⁻¹ of N (as urea) were applied as side-

152 dressing fertilization, during the 25th and the 16th of May respectively in 2013 and 2014.

153 Irrigation was conducted using the pivot method, providing each time 50 mm of water, in

154 order to avoid any drought stress until the end of the dough stage (GS 87). Irrigation was
155 performed twice in 2013, whereas it was only applied once in 2014, due to abundant
156 rainfall in the summer. Weed control was conducted at pre-emergence with mesotrione
157 (0.15 kg AI ha⁻¹) S-metolachlor (1.25 kg AI ha⁻¹) and terbuthylazine (0.75 kg AI ha⁻¹)
158 (Lumax[®], Syngenta Crop Protection S.p.A., Milan, Italy). All the plots were sprayed at GS
159 75 in both trials with pyrethroid lambda-cyhalothrin insecticide (Karate[®] Zeon, Syngenta
160 Crop Protection S.p.A., Milan, Italy) at 0.019 kg AI ha⁻¹. The spraying application was
161 performed on July 19th and 22nd, respectively, for the 2013 and 2014 growing seasons.
162 This allowed any possible damage to the stalks and ears, due to the larva activity of the
163 European Corn Borer (ECB) (*Ostrinia nubilalis* Hübner), to be restrained.

164 **2.1.2 Experiment 2**

165 The standard planting density (StD) was compared against the innovative high planting
166 density (HiD) for several production situations (PS) (Aubertot and Robin, 2013) from the
167 year 2011 to 2014. The StD involved the ordinary planting pattern and density that are
168 commonly adopted in the maize temperate growing areas of Europe and North America.
169 This consists of an average seeding rate of 7.5 plants m⁻², planted in rows 0.75 m apart,
170 with an average intra-row spacing of 0.18 m. The HiD system had the purpose of
171 maximizing grain yield by increasing the seeding rate to 10.5 plants m⁻². In order to
172 achieve this density, and at the same time to keep plants as equidistant as possible, the
173 distance between rows was reduced to 0.5 m. The average gap between one plant and
174 another was set to 0.19 m within each row. On both systems, planting was conducted by a
175 mechanical sowing machine with adjustable row width. All seed were treated with
176 fludioxonil and metalaxil-m (Celest XL[®], Syngenta Crop Protection S.p.A., Milan, Italy).
177 Overall, the comparisons between the StD and HiD systems were made considering 34
178 PS, according to a completely randomized block design with 4 replicates. All the plots

179 were 10 m long and 8 or 12 rows wide, for the StD and HiD systems, respectively. All the
180 measurements were conducted on the two middle rows. The list of the compared PS and
181 their main geographical and agronomical information is reported on Table 1.

182 A 0.3 m deep ploughing and an insecticide treatment were applied at GS 75 in all the trial
183 fields in order to restrain the ECB activity. All the hybrids in the comparison belonged to a
184 full maturity class (FAO 500 or 600) and they are representative of the varieties more
185 cultivated in the considered growing area. As described in Table 1, different irrigation
186 systems were adopted, according to the typical farm management system in force in the
187 area, each providing enough water to maintain the water-holding capacity between 33 and
188 200 kPa. Other agronomical practices that have not been reported, such as fertilization
189 and weed control, were conducted according to the ordinary agronomic techniques of the
190 cultivation area and were the same for both density theses.

191 **2.2 Field and harvest assessments**

192 **2.2.1 Experiment 1**

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

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

203
$$(1) \text{ Stalk area (cm}^2\text{)} = \left(\frac{D \text{ (mm)}}{2} * \frac{d \text{ (mm)}}{2} * \pi \right) / 100$$

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

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

207 At the end of maturity, the grain yields were obtained by harvesting the entire length of the
208 2 middle rows in the 0.75 m SIS plots and the 3 middle rows in the NIS plots using a
209 Walter Wintersteiger cereal plot combine-harvester. The grain yield results were adjusted
210 to a 14% moisture content. This operation was carried out on October 15th and October 6th
211 for the 2013 and 2014 growing seasons, respectively.

212 Before the mechanical harvest, ears were collected by hand and counted from each plot,
213 considering a surface of 4.5 m², in order to record the number of lodged plants, to
214 calculate the density per square meter of the fully developed ears (ESQ) and to conduct
215 further assessments regarding the morphology of the ears and kernels.

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

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

224 The grain moisture and the test weight (TW) were determined on wet and dry kernels,
225 respectively, by means of a Dickey-John GAC2000 grain analysis meter (Dickey-John
226 Corp. Auburn, IL, USA) according to the supplied programme. Two hundred dry kernels
227 were randomly collected and weighed to assess the thousand kernel weight (TKW). The

228 kernel volume (KV) was measured by recording the rise in the known alcohol volume after
229 inserting 100 kernels into a graduate cylinder. Two analytical replicates were carried out
230 for all these assessments. The average kernel length (KL) and kernel depth (KD) was
231 measured on 30 kernels randomly selected from each sample using a digital gauge.

232 **2.2.2 Experiment 2**

233 At the end of maturity, ears were collected manually from an area of 4.5 m² in each plot,
234 weighed, counted and then shelled. The following assessments were conducted on the
235 kernels, according to the same procedure as experiment 1: grain moisture, TW, TKW, KR,
236 KPR and KSQ. The ear weight (including the cob and grains) was calculated as the total
237 weight of the ears divided by the number of harvested ears.

238 **2.3 Statistical analysis**

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

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

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

250 Multiple comparison tests were performed, according to the Ryan-Einot-Gabriel-Welsch F
251 (REGW-F) test, on the planting density treatment means. SPSS Version 21.0 for Windows

252 statistical package was used for the ANOVA statistical analysis.

253 The relative ratio (RR= HiD/StD) between the HiD agronomic system and StD was

254 calculated for each measured parameter for all the PS considered in experiment n° 2. The

255 average RR and the confidence interval were calculated at significance levels of 99.9%

256 (P<0.001), 99% (P<0.01) and 95% (P<0.05).

257 **3. Results**

258 **3.1 Experiment 1**

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

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

269 The average cob and tipback length of the fixed hybrid resulted to be longer than the flex
270 one, that is, +6.7 and +9.9%, respectively. The KPR was also higher, while the KSQ was
271 significantly lower (-13.4%), and this was mainly due to a reduced KR (15.7 rows versus
272 19.4). The KV was also higher (+5.4%) as was the TKW (+4.7%) and KL (+3.5%), whereas
273 the KD was lower (-6.6%).

274 Of all the parameters related to plant architecture, the ones that were influenced the most
275 by inter-row spacing were the stalk area and ear height. The plants in the narrow row
276 system (NIS) had a thicker stalk (4.1 versus 3.7 cm², +8.9%) but also a slightly higher (+5
277 cm) ear height. The planting density significantly influenced the stalk development and ear
278 height, but also the leaf greenness during the ripening stage, the latter being an indicator
279 of the nutritional status of the crop. The reduction in the stalk area from the lowest
280 population density (D1: 7.5 plants m⁻²) to the highest (D4: 12 plants m⁻²) was of 20%,

281 whereas it was -5.2% for leaf greenness, which was expressed as an NHT value. Even
282 though the increased planting population led to thinner plants, which were also
283 characterized by a taller ear height, none of the compared treatments displayed a lodging
284 propensity, and plant lodging was lower than 2% for both of the considered growing
285 seasons. The interactions between inter-row space and planting density for these plant
286 morphological parameters were never significant.

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

301 As expected, the average number of ears harvested per unit area (ESQ) significantly
302 increased as the growing density increased. This parameter resulted to be 8.9% higher in
303 the NIS than in the SIS, which also explains the 9.9% KSQ difference between the
304 compared inter-row spacing systems. Figure 2 reports the ESQ values for the different
305 planting densities for the NIS and the SIS. Only in the D1 thesis were the ESQ values from

306 both inter-row planting patterns close to the target planting density of 7.5 plants m⁻² (7.7
307 ears m⁻² for the NIS and 7.2 ears m⁻² for the SIS). For the higher plant densities, the
308 number of ears harvested per unit area was on average lower than the number of plants
309 per unit area, foreseen in the experimental protocol. The gap between the harvested ears
310 (ESQ) and the number of plants per unit area resulted to gradually rise as the planting
311 density was increased. Furthermore, this trend was clearly more consistent for the SIS
312 than for the NIS: the average ESQ in the D4 thesis (12 plants m⁻²) for the NIS was 11.3
313 ears m⁻² and it was significantly different from the D4 thesis for the SIS, which resulted in
314 an average of 10 ears m⁻². The same trend was observed for the D3 thesis: an average of
315 10.2 ears m⁻² were harvested for the NIS, whereas only 9.1 ears m⁻² were harvested for
316 the SIS.

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

323 No significant differences were observed for grain yield between NIS and SIS at the lower
324 plant density (D1 and D2). However, the NIS resulted in a significantly greater grain yield
325 (+10.9%) than the SIS for the higher plant densities (D3 and D4).

326 The different inter-row spacing did not influence any of the kernel parameters, that is,
327 moisture, TW, TKW, KL, KD or KV. On the other hand, the more intense plant population
328 negatively influenced the length, volume and weight of the kernels. The TKW in fact
329 diminished by 7.1% for between 7.5 and 12 plants m⁻², whereas the KV diminished by
330 5.7%. The interactions between the inter-row spacing and planting density were never

331 significant for the kernel parameters.

332 **3.2 Experiment 2**

333 The results of the application of the innovative high plant density system (HiD), compared
334 to the standard one (StD), in the 34 production situations (PS), are reported in Table 4.

335 The average yield increase obtained by switching from StD to the HiD was of 1.8 t ha⁻¹
336 (+11%, P<0.001). The grain moisture, TW and KR were not affected by the two different
337 seeding rates. Conversely, the TKW decreased by 5.7% from the StD to the HiD. The
338 average ear weight dropped by 18% after the adoption of the innovative system. The same
339 trend, but less pronounced (-9%) was seen for the KPR value. However, because of the
340 higher number of ears per square meter, the total amount of kernels per square meter
341 (KSQ) increased (+23%).

342 Figure 4 represents the percentage of grain yield variation obtained after adopting the HiD
343 system, sorted from the lowest to the highest in all 32 PS taken into account. Only 6.2% of
344 the cases (2 PS out of 32) showed a negative effect, with an average yield decrease of
345 6.8%, while one PS showed no variations. The remaining cases (90%) instead showed a
346 yield increase of between 1.3% and 22%.

347

4. Discussion

348

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

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

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

372 As far as the kernel dimension is concerned, the increase in plant density caused a

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

381 All the previously discussed aspects confirm that compared to the standard plant density
382 for full maturity hybrids a higher planting population, which implies fewer, smaller and
383 lighter kernels per ear, lowers the yield potential of the single maize plant (Li et al., 2015).
384 As can clearly be seen from the multiple comparison of experiment 2 (Table 4), the
385 average ear weight decreased by 18% for the high plant density system. However, the key
386 to the yield enhancement obtained from the application of a high plant population in
387 modern hybrids, is the higher number of kernels harvested per unit area (KSQ), which was
388 +26% and +23% for experiment n° 1 and 2, respectively, comparing 7.5 and 10.5 plants
389 ha⁻¹. For highly competitive conditions, which are typical of high plant populations, the final
390 grain yield is linked more to the number of harvested kernels than to the kernel weight
391 itself (Sangoi et al., 2002). In the environmental conditions in which experiment 1 was
392 conducted, a significant yield increase for a higher plant population (10.5 plant m⁻²) was
393 only observed in NIS (0.5 m inter-row distance), and not in the SIS (0.75 m inter-row
394 distance) (Figure 3). The main advantage of the NIS was that a higher number of ears per
395 area unit (ESQ) was harvested than for SIS (Figure 2). In fact, the plants grown with a high
396 plant density in the SIS had a higher incidence of barrenness than those grown in the NIS.
397 This is the consequence of the lower equidistance space that was adopted in the SIS: by

398 maintaining the 0.75 m inter-row width and moving from 7.5 plants m⁻² to 12 plants m⁻², the
399 average distance of two contiguous plants on the same row was reduced from 0.18 m to
400 0.11 m. With a high increase in the plant population for a large inter-row spacing, the risk
401 of having higher irregularities of the plant distance in the row is greater and consequently
402 the percentage of dominated plants, which are often infertile is also higher (Lauer and
403 Rankin, 2004). However, the NIS system allows a more even distance between intra-row
404 plants and thus alleviates intercrop competition, therefore only in this condition, a reduced
405 plant spacing, yet more equidistant, allowed a significant yield increase. The better
406 response of NIS has confirmed what was observed in previous studies on silage maize
407 (Cox and Cherney, 2001). Farnham (2001) also reported a better yield increase when
408 adopting higher planting populations for an even narrower (0.38 m) inter-row space,
409 although the average grain yield was higher for a 0.76 m wide one. In other studies in
410 which the highest plant density tested was 10 plants m⁻² (Nielsen, 1988; Porter et al.,
411 1997; Widdicombe and Thelen, 2002), it was reported that row width was inversely
412 correlated to the grain yield and that an interaction between plant density and row width
413 was observed. The use of light, with a better interception of the solar radiation, is more
414 efficient on narrow rows, and contributes by maximizing the final yield (Onyango, 2009).
415 This type of planting leads to a better shading of the soil surface during the early part of
416 the season, and limits water evaporation from the bare soil, as reported by Bullock et al.
417 (1998) for soybean. Moreover, a more shaded inter-row space does not offer a suitable
418 environment for weeds. The NIS in fact could reduce competition with weeds through early
419 canopy closure (Fanadzo et al., 2010). This planting pattern, although resulting in a more
420 enhanced yield when a high planting density is adopted, needs to be supported with
421 appropriate field equipment. The tractor wheels and track base width have to be
422 dimensioned correctly as well as the combine harvester header and harrow squadrons, in

423 order to prevent any driving over plants. Furthermore, it is expected that the inter-row
424 tillage in a NIS would be shallower, thus limiting soil ventilation, water penetration and
425 mechanic weed control. However, no further difficulties appeared for the irrigation process,
426 in either the furrow inter-row method, or in the over canopy ones (sprinkling/pivot
427 systems).

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

432 The highest planting density sown at 12 plants m^{-2} was not sufficient to determine a yield
433 breakdown of either the inter-row spacing or the hybrids. However, this planting density
434 treatment did not significantly differ from when the plants were sown at a rate of 10.5
435 plants m^{-2} (Figure 3), thus confirming that, for the agronomical conditions that were tested,
436 the best crop yield potential was obtained for 10.5 plants m^{-2} . This work has shown that, in
437 growing areas which are suitable for maize cultivation, under irrigated and fertile
438 conditions, an increase in planting density from 7.5 to 10.5 plants m^{-2} with the present full
439 season hybrids represents a real opportunity for farmers to boost the crop yield potential.
440 This agronomical strategy, albeit very promising, needs to be considered carefully in the
441 different production situations, since the risk of losses related to greater stress can
442 increase. As far as plant stand ability is concerned, the stalk section was shown to be
443 negatively affected by the higher planting population (Table 3), whereas the ear height had
444 an opposite trend. Plants sown more densely, receive a different quality of light,
445 characterized by enriched far red and impoverished red radiation (Rajcan and Swanton,
446 2001). This can in fact stimulate apical dominance, and induce plants to grow taller, with a
447 thinner stalk that is more disposed to lodging or breaking (Sangoi et al., 2002; Stanger and

448 Lauer, 2007; Widdicombe and Thelen, 2002). Even though ear insertion tended to be
449 higher for the NIS, the stalk area was also larger, and hence more tolerant to plant lodging.
450 Moreover, it has also been reported for maize, that some chemical compounds, known as
451 plant growth regulators (PGRs), can modify plant growth by reducing height and thus
452 lodging risks when a high planting density is applied or in other conditions (Shekoofa and
453 Emam, 2008; Zhang et al., 2014). In the fields where the trials were carried out, lodging
454 was not observed to be connected to any treatment. Similar results have also been
455 recorded in experiments conducted on lodging tolerant hybrids in Brazil (Sangoi et al.,
456 2002) on densities of 10 plants m⁻².

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

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

487

488 **5. Conclusion**

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

496

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613

614 **TABLES**

615

616 **Table 1.** List of production situations (PS) in which experiment 2 was carried out. Year, location,
 617 growing degree days (GDD) from April to October, soil texture, hybrid, and planting and harvest
 618 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

619

620 ^a GPS coordinates for each location: Carignano N44°52'48" E7°37'42"; Carmagnola N44°53'11" E7°41'15";

621 Verolengo N45°13'47" E7°56'41"; Buriasco N44°51'48" E7°26'20"; Macello N44°51'21" E7°25'44"; Vigone

622 N44°50'19" E7°28'55"; Chivasso N45°12'42" E7°55'41".

623

624 **Table 2.** Monthly total rainfall, rainy days, average temperature and growing degree days (GDD)
 625 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)
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

626

627

628 **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,
629 kernel rows (KR), kernels per row (KPR), kernels per square meter (KSQ), ears per square meter (ESQ), grain yield, grain moisture, test weight
630 (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 greenness (NHT value)	Cob length (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

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

632 ^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
633 maturity class 500; Flex hybrid: KWS Korimbos, FAO maturity class 500.

634 ^b NS, not significant (P(F)<0.05).

635 ^cSEM, standard error of means.

636 ^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,
637 while the standard inter-row (SIS) is 0.75 m wide.

638 ^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
639 plants m⁻²; D4: 12 plants m⁻².

640

641 **Table 4.** Comparison of the innovative planting density (HiD) and the standard density (StD)
 642 in different production situations^a on grain yield, grain moisture, test weight (TW), thousand
 643 kernel weight (TKW), kernel rows (KR), kernels per row (KPR) and kernels per square meter
 644 (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	***

645

646 ^a The production situations refer to those reported in table 1.

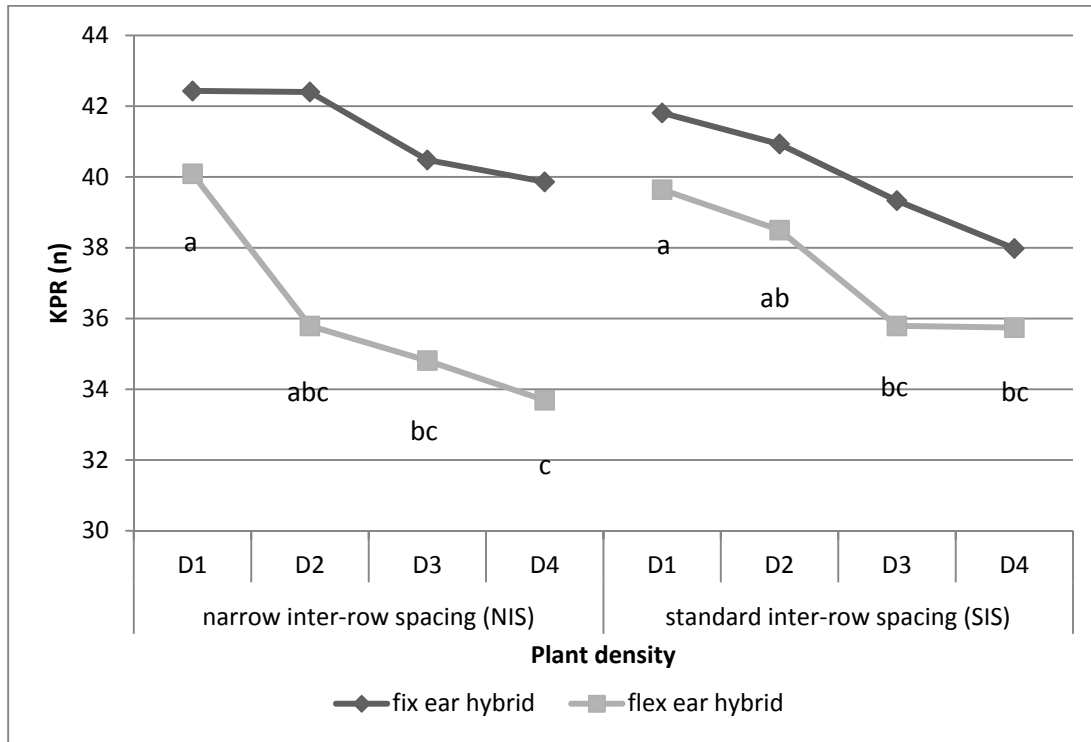
647 ^b The reported values are mean for each treatment, relative ratio (RR = HiD/StD) and significance in
 648 term of interval of confidence.

649 ^c NS = not significant at P<0.05.

650

651

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



654

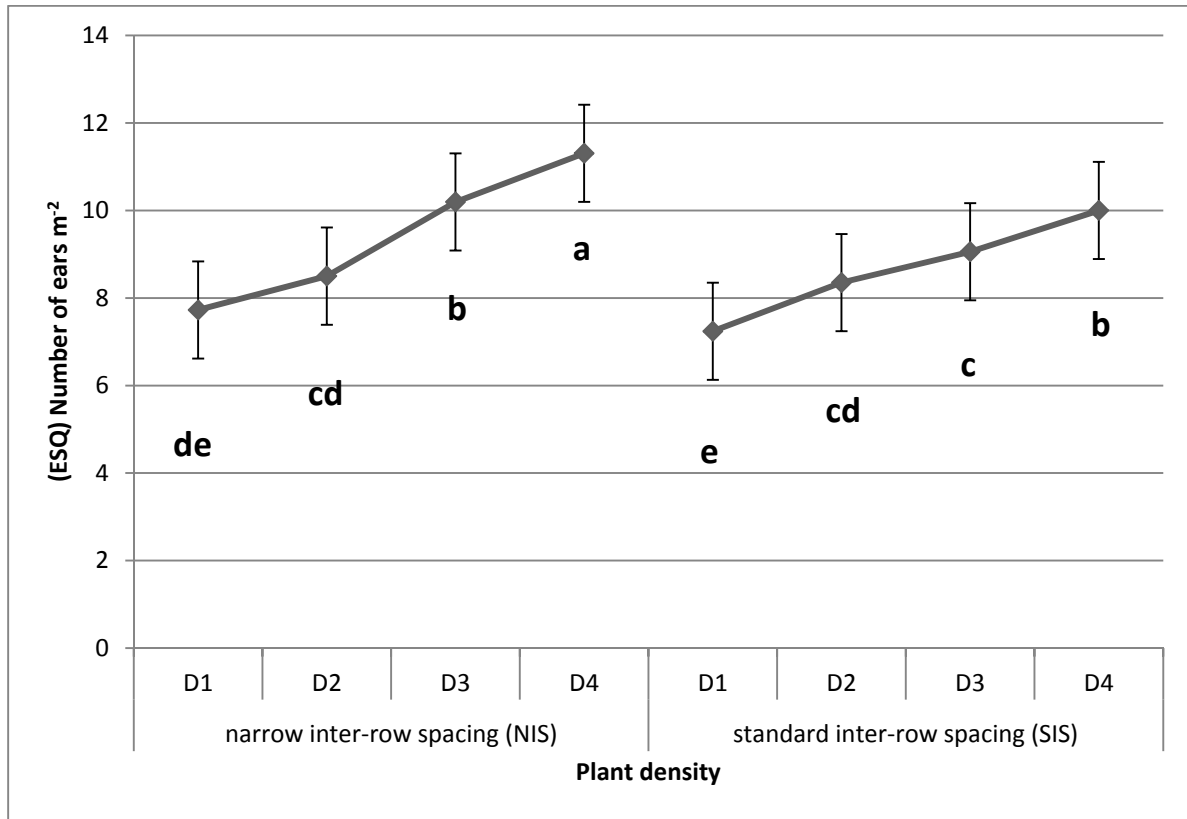
655 Means of the flex ear hybrid followed by different letters are significantly different for $P < 0.001$ and a
 656 standard error of means (SEM) of 1.46. No significant differences were observed for the fixed ear
 657 hybrid (SEM=3.4).

658 The planting density X inter-row spacing values for each hybrid are based on 6 repetitions (2 years X
 659 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} ;
 660 D2 9 plants m^{-2} ; D3 10.5 plants m^{-2} ; D4 12 plants m^{-2} .

661 Fix hybrid: Syngenta NX7234, FAO maturity class 500; Flex hybrid: KWS Korimbos, FAO maturity class
 662 500.

663

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



666

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

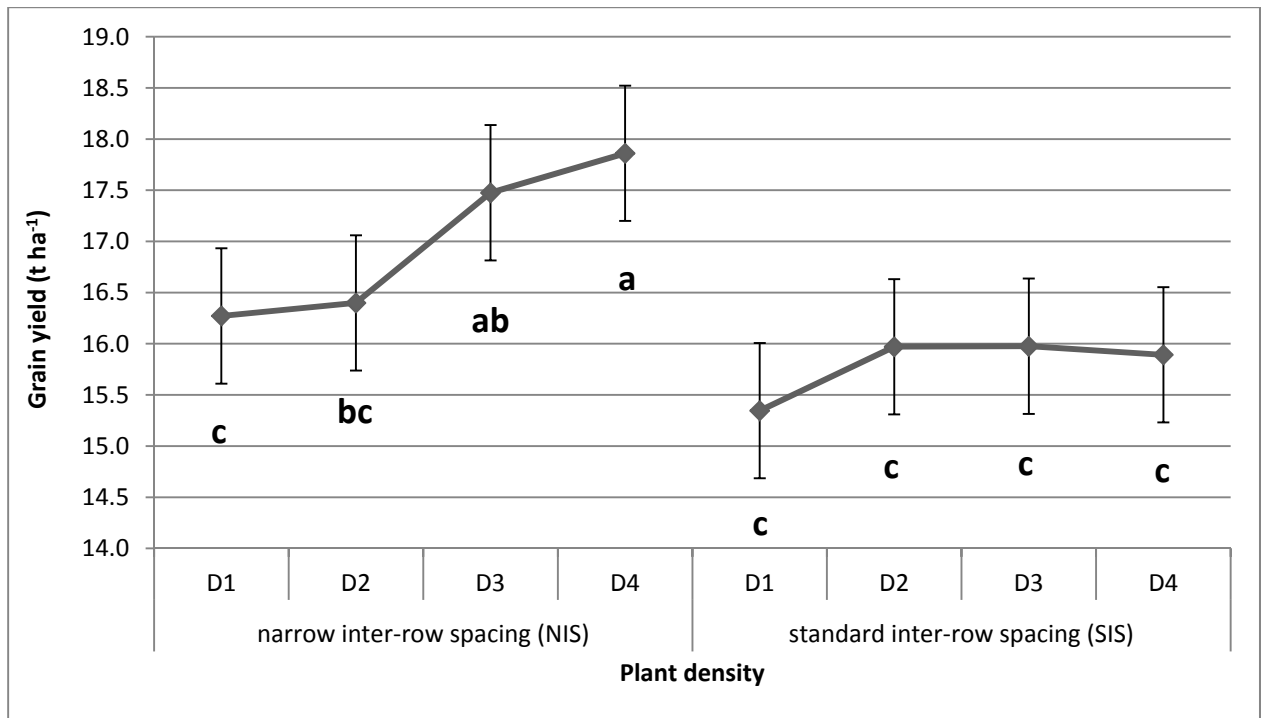
668 Inter-row spacing X planting density values are based on 12 repetitions (2 years X 2 hybrids X 3
 669 replicates). The narrow inter-row was 0.5 m wide, while the standard inter-row was 0.75 m wide.

670 Plant density: D1 7.5 plants m⁻²; D2 9 plants m⁻²; D3 10.5 plants m⁻²; D4 12 plants m⁻².

671

672

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



674

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

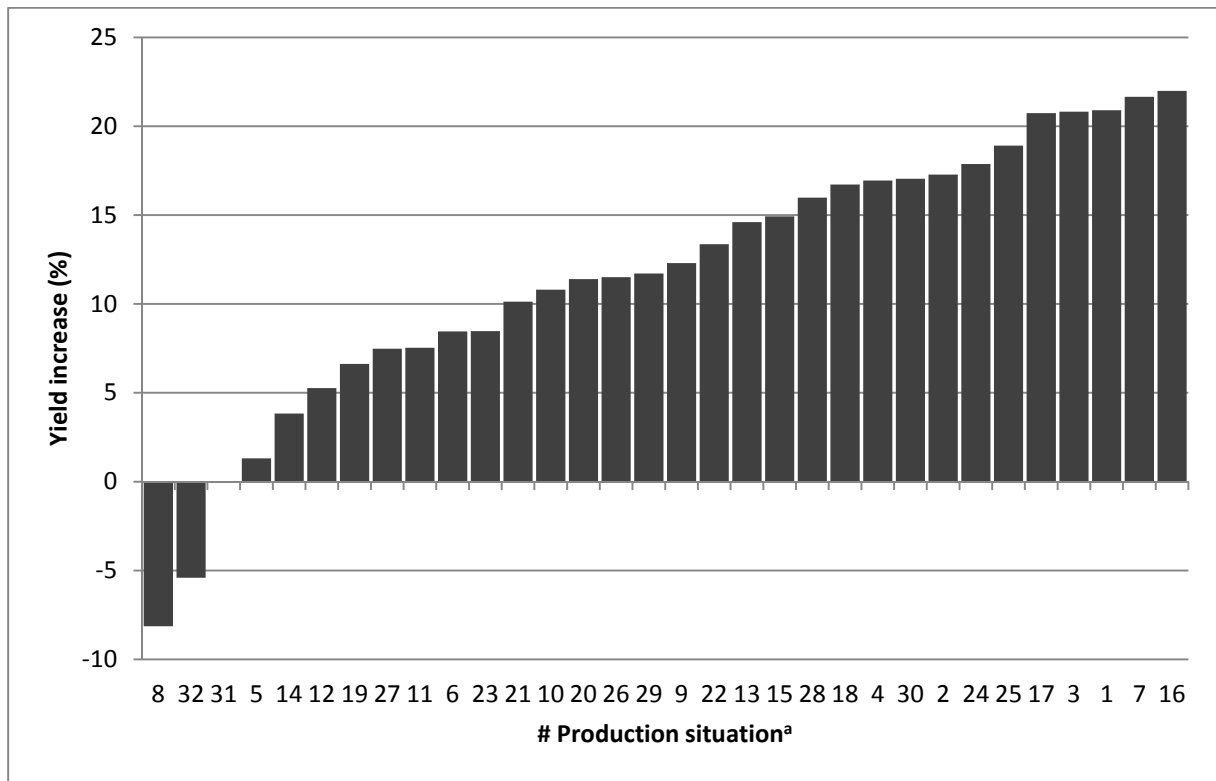
676 Inter-row spacing X planting density values are based on 12 repetitions (2 years X 2 hybrids X 3
677 replicates). The narrow inter-row was 0.5 m wide, while the standard inter-row was 0.75 m wide.

678 Plant density: D1 7.5 plants m⁻²; D2 9 plants m⁻²; D3 10.5 plants m⁻²; D4 12 plants m⁻².

679

680

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



683

684 ^a The production situations refer to those reported in table 1.