1 **Strategies of grain number determination differentiate barley row-**

2 **types**

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22 **Highlight**

23 In cereals, understanding the interactions of different yield components that influence

24 the grain number is essential to increase their yield by modulating the components.

25 We show in this study that the grain number of two-rowed barley is predominantly

26 determined by the potential spikelet number while in six-rowed by spikelet survival.

27 **Abstract**

28 Gaining knowledge on intrinsic interactions of various yield components is crucial to
29 improve the yield potential in small grain cereals. It is well known in barley that
30 increasing the grain number (GN) preponderantly improves their yield potential;
31 however, the yield components determining GN and their association in barley row-
32 types are less explored. In this study, we assessed different yield components like
33 potential spikelet number (PSN), spikelet survival (SSL), spikelet number (SN), grain
34 set (GS), and grain survival (GSL), as well as their interactions with GN by using a
35 selected panel of two- and six-rowed barley types. Also, to analyze the stability of
36 these interactions, we performed the study in two growth conditions, greenhouse and
37 field. From this study, we found that in two-rowed, GN determination is strongly
38 influenced by PSN rather than SSL and/or GS in both growth conditions. Conversely,
39 in six-rowed, GN is associated with SSL instead of PSN and/or GS. Thus, our study
40 exemplified that increasing GN might be possible by augmenting PSN in two-rowed
41 genotypes, while for six-rowed genotypes, the ability of SSL needs to be improved.
42 We speculate that this disparity of GN determination in barley row-types might be
43 due to the fertility of lateral spikelets. Collectively, this study revealed that the GN of
44 two-rowed largely depends on the developmental trait, PSN, while in six-rowed, it
45 mainly follows the ability of SSL.

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48 **Keywords:** Grain yield, Grain number determination, Maximum yield potential,
49 Potential spikelets, Spikelet survival, Barley.

50 **Introduction**

51 Propelling the crop yield is one of the essential aims in the field of plant sciences with

52 the limitations of climatic change and declining arable land. However, crop yield is a

53 complex trait influenced by many abiotic and biotic factors, as well as different plant

54 developmental traits. Barley (*Hordeum vulgare* L.) is the fourth economically

55 important cereal crop in the world (FAOSTAT, 2019). It stands as a model crop of the

56 tribe Triticeae, which contains other major cereals: wheat (*Triticum* spp.), rye (*Secale*

57 *cereale* L.), and triticale (x *Triticosecale* Wittm.) (Ullrich, 2011; Mascher et al., 2017).

58 Triticeae species have a characteristic unbranched inflorescence called the spike, on

59 which the floral units called spikelets are arranged in a distichous pattern. Barley

60 produces three spikelets (one central and two lateral) per inflorescence node, and

61 the spikelets are determinate that they form a single floret per spikelet. Whereas,

62 wheat forms one spikelet per node, while its spikelet develops florets indefinitely

63 without limited by a terminal floret since wheat spikelet is indeterminate (Bonnett,

64 1966; Kirby and Appleyard, 1984; Koppolu and Schnurbusch, 2019). Based on the

65 fertility of the lateral spikelets, barley is commonly classified as two-rowed (both the

66 lateral spikelets are sterile and form no grains) and six-rowed (all three spikelets are

67 fertile and form grains). In principle, six-rowed types produce three times more grains

68 per spike than a two-rowed spike. The major gene responsible for the row-type

69 difference is known as *Six-rowed spike 1* (*Vrs1*) that affects the pistil development of

70 the lateral spikelets (Komatsuda et al., 2007; Sakuma et al., 2013; Zwirek et al.,

71 2018). Even though two-rowed lateral spikelets do not set grains, they are retained

72 as partially developed spikelets on the two-rowed spike.

73

74 In the field of barley and hexaploid wheat (*Triticum aestivum* L.), efforts have been

75 undertaken to unravel the impact of many traits like spike biomass, floret survival,

76 crop growth rate, ovary size, grain number, and grain set on grain yield

77 (Arisnabarreta and Miralles, 2006b; Arisnabarreta and Miralles, 2008a; Sadras and

78 Slafer, 2012; Serrago et al., 2013; Alqudah and Schnurbusch, 2014; Arisnabarreta

79 and Miralles, 2015; González-Navarro et al., 2015; Guo and Schnurbusch, 2015;

80 Slafer et al., 2015; Gonzalez-Navarro et al., 2016; Guo et al., 2016; Garcia et al.,

81 2019). Grain yield is majorly composed of grain number (GN) per unit area and grain

82 weight, and it is well documented that GN is the most dominant trait influencing yield

83 in barley and wheat (Prystupa et al., 2004; Peltonen-Sainio et al., 2007; Ugarte et al.,

4

84   2007; Serrago et al., 2013; García et al., 2015; Sakuma and Schnurbusch, 2020). In

85   these crops, the GN is determined by the number of fertile florets (wheat) or spikelets

86   (barley) at anthesis. In general, three critical phases of the spikelet initiation and

87   growth explains the whole process of a spikelet to grain transformation. The first

88   phase is the floret/spikelet initiation phase that starts with the initiation of

89   florets/spikelets and ends with the maximum number of florets/spikelets (also known

90   as potential florets/spikelets). The end of the floret/spikelet initiation phase is known

91   as the maximum yield potential stage (MYP). The phase following the MYP is the

92   spike growth phase that overlaps with the stem elongation period. During this phase,

93   the final number of florets/spikelets are determined after their abortion. The spike

94   growth phase ends at or around the anthesis stage when the final fertile

95   floret/spikelet number is attained. After these two phases, the fertile florets/spikelets

96   enter the grain set phase during which the final GN is reached by filling the stored

97   assimilates to the fertile florets/spikelets (Gallagher, 1979; Sreenivasulu and

98   Schnurbusch, 2012).

99

100   From the available literature on wheat yield and yield components, we found two

101   proposed models that determine the number of fertile florets or GN. The first and the

102   most popular one is the 'survival model' which claims that the number of fertile florets

103   is determined by floret survival, regulated through the assimilate allocation to the

104   growing spike (Fischer and Stockman, 1980; Kirby, 1988; Ghiglione et al., 2008;

105   Gonzalez et al., 2011; Ferrante et al., 2013). The other hypothesis challenged the

106   survival model and proposed a 'developmental model' in which GN has a stronger

107   correlation to the time of floret death, which is a pure developmental process. It was

108   also shown that the onset of floret abortion is loosely associated with spike growth;

109   instead, it is determined by the developmental stage of the most advanced floret of

110   the spike (Bancal, 2008, 2009). Concertedly, the survival model, strongly proposes

111   that the second phase, spikelet growth, define GN. In contrast, the developmental

112   model supports the first spikelet initiation phase, which determines the final GN.

113   Many studies in barley explored different yield components and their interaction with

114   GN (del Moral et al., 1991; Prystupa et al., 2004; Ugarte et al., 2007; García et al.,

115   2015; Kennedy et al., 2018). Also, these interactions were compared between the

116   two- and six-rowed types (del Moral et al., 2003; Arisnabarreta and Miralles, 2006a,

117   2008b; Arisnabarreta and Miralles, 2008a, 2015). However, to our knowledge, there

118　are no comprehensive studies that compared the interaction of the three phases of

119　the spikelet initiation and growth and their interaction to the final GN in two- and six-

120　rowed barley types. This knowledge gap must be addressed because both the row-

121　types have a different pattern of spikelet initiation and growth and uniformity of grain

122　size and content (Kirby and Riggs, 1978; Riggs and Kirby, 1978; Frégeau-Reid et al.,

123　2001; Zwirek et al., 2018; Dodig et al., 2019; Kandic et al., 2019).

124

125　In this study, we explored the interaction of the critical yield components like potential

126　spikelet number (PSN), spikelet survival (SSL), and grain set (GS) with GN from the

127　main culm of barley row-types. Also, we analyzed the association of a few more traits

128　(from the main culm), such as spikelet number (SN) at harvest and grain survival

129　(GSL) with GN to understand their impact on the final GN in barley. We compared

130　these interactions between the barley row-types, two- and six-rowed, which were

131　grown in the greenhouse and field. Interestingly, two-rowed types performed

132　consistently better in the field for all the traits except PSN, while in six-rowed only GN

133　and GSL were improved. The interactions of PSN, SSL and GS with GN in both

134　growth conditions clearly distinguished the row-types and revealed that the two-

135　rowed GN strongly depends on PSN rather than SSL and GS. Contrastingly, in six-

136　rowed, GN is majorly influenced by SSL than PSN and GS. The GS in six-rowed

137　exhibited a much higher impact on GN in the greenhouse than in the field, while in

138　two-rowed GN is less affected by GS in both the environments. Collectively, from this

139　study, we concluded that barley row-types are distinct in their yield components

140　interaction with GN. Specifically, in two-rowed, GN depends on the spikelet initiation

141　trait, PSN, while in six-rowed, it is majorly influenced by the spikelet growth trait, SSL.

142

143　**Materials and Methods**

144　**Plants and growth conditions**

145　The study was carried out at the Leibniz Institute of Plant Genetics and Crop Plant

146　Research, Gatersleben, Germany (51° 49′ 23″ N, 11° 17′ 13″ E, altitude 112 m)

147　during the 2017 growing season. Twenty-seven barley accessions (Supplementary

148　Table 1) were selected from a worldwide collection panel (Alqudah et al., 2014)

149　based on their variation in spike developmental traits. The selected panel was used

150　for two experiments conducted in the field (April to August 2017) and greenhouse

151　(July to December 2017). For both the experiments, similar sowing and pre-treatment

152   protocols were followed. Barley grains were sown on 96 well trays and grown under
153   greenhouse conditions (photoperiod, 16h/8h, light/dark; temperature, 20° C/16° C,
154   light/dark) for two weeks. Plants were vernalized at 4° C for four weeks and then
155   acclimatized in the greenhouse for a week. Following the hardening process, plants
156   were directly transplanted in the silty loam soil for the field experiment. The field
157   experiment was a single plot per genotype, and each plot had eight rows with a 15
158   cm distance between the rows. Each row was 0.8 m long with five plants per row. In
159   the greenhouse experiment (photoperiod, 16h/8h, light/dark; temperature, 20° C/16°
160   C, light/dark), plants were potted in a 9 cm pot (9 × 9 cm, diameter × height) with two
161   parts of autoclaved compost, two parts of 'Rotes Substrat' (Klasmann-Deilmann
162   GmbH, Germany), and one part of sand. We conducted a third experiment in the field
163   (same location mentioned above) named 'Random culm-field' between April 2019 to
164   August 2019. In this experiment, the 27 genotypes were replicated in three random
165   block model, in which a block size was 1 × 1.5 m^2, and in every block, a genotype
166   was sown with a density of 300 grains/m^2. Each block had six rows spaced 20 cm
167   between them. Standard practices for irrigation, fertilization, and control of pests and
168   diseases were followed. The temperature, light, and other environmental conditions
169   of the field, greenhouse, and random culm-field experiments are given in
170   supplementary table 2.

171

172   **Phenotyping traits and measurement**
173   All the traits reported from the field and greenhouse studies (the year 2017) were
174   measured from the main culm of a barley plant because of its higher contribution to
175   the final grain yield and it is less influenced by environmental perturbations and
176   growth conditions (Cottrell et al., 1985; Elhani et al., 2007). The direct yield
177   component traits like potential spikelet number (PSN), final spikelet number (SN),
178   and grain number (GN) were collected from randomly selected plants at two stages,
179   maximum yield potential (MYP) and harvest. PSN was counted at the MYP stage,
180   while SN and GN were enumerated at the harvest. At the MYP stage, data were
181   collected from three random plants for both the experiments, whereas at harvest, six
182   plants were used in the field and twelve plants in the greenhouse experiment.
183   However, in the random culm-field experiment, all the parameters were collected
184   from three random culms of every block, which made nine replications per genotype.
185   The MYP stage was identified by tracking the development of spikes based on the

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186 developmental scales previously been used (Waddington, 1983; Kirby and

187 Appleyard, 1984). The stage at which the initiation of spikelet primordia stops at the

188 apex of the main shoot spike was considered as the MYP stage.

189 All the initiated spikelet primordia and differentiated spikelets were counted at the

190 MYP stage and regarded as potential spikelet number (PSN) of a spike. At MYP,

191 more apically localized spikelet primordia ridges in the double ridge stage were

192 multiplied by three since barley spikes form three spikelet primordia at a spike axis

193 (rachis) node (Waddington, 1983; Kirby and Appleyard, 1984). The final SN and GN

194 were counted from a spike after the physiological maturity of the plant. All

195 (assimilate-) filled and unfilled spikelets were counted as final SN, while only the filled

196 spikelets were regarded as grains. Spikelet and grain survival traits were calculated

197 from the equations (1) and (2), respectively, and grain set from equation (3).

198

$$Spikelet\ survival = \frac{Final\ spikelet\ number}{Potential\ spikelet\ number} \times 100 ------ (1)$$

199

$$Grain\ survival = \frac{Grain\ number}{Potential\ spikelet\ number} \times 100 -------- (2)$$

200

$$Grain\ set = \frac{Grain\ number}{Final\ spikelet\ number} \times 100 -------- (3)$$

201

Methods of data analysis

203 All the data analyzes were done in the Prism software, version 8.4.2 (GraphPad

204 Software, LLC), except the non-linear quadratic (squared) fit. The outliers were

205 identified by the 'ROUT' method described previously (Motulsky and Brown, 2006).

206 The mean value comparison between the environments for every genotype and row-

207 types was analyzed by the multiple t-tests and paired t-test (parametric), respectively.

208 The false discovery rate approach of the two-stage linear step-up procedure of

209 Benjamini, Kreier, and Yekutieli with the Q value of 5% was applied to calculate the

210 significance of t-tests. Only for PSN and GS, a two-way ANOVA with Tukey's multiple

211 comparison test (alpha=5%) was conducted. The replicate values of a genotype and

212 the row-types were analyzed individually, without assuming a consistent standard

213 deviation. Linear regression was done using the appropriate dependent (Y values)

214 and independent (X values) traits. The 95% confidence intervals were identified for

8

215   every linear regression and plotted as confidence bands along with the 'goodness of

216   fit' line. Non-linear quadratic (squared) fit for selected dependent and independent

217   traits was done with the data analysis tool kit of Microsoft office-Excel (version 2016).

218

219   **Results**

220   **Potential spikelet number is influenced by the combination of genetic and**

221   **environmental variations**

222   Previous studies in barley did not consider lateral spikelet primordia and spikelets of

223   two-rowed barleys as "potential spikelets" for the determination of MYP

224   (Arisnabarreta and Miralles, 2004, 2006b). However, after the discovery of several

225   *Vrs1* alleles (Komatsuda et al., 2007; Sakuma et al., 2017; Casas et al., 2018) and

226   various row-type genes that influence lateral spikelet initiation and growth (Ramsay

227   et al., 2011; Koppolu et al., 2013; Bull et al., 2017; van Esse et al., 2017; Youssef et

228   al., 2017; Zwirek et al., 2018), it was clearly shown that the two-rowed lateral

229   spikelets, though sterile, also influence grain size of the central spikelets. To account

230   for this deficiency, we included lateral spikelet primordia and spikelets of two-rowed

231   types as potential spikelets besides the central spikelets for main culm MYP

232   determination. Within the selected 17 two-rowed types, the genotype BCC1705

233   developed the lowest PSN (75±0) in the greenhouse, while BCC929 produced the

234   highest PSN (140±1.7) in the field. Comparably, among the ten chosen six-rowed

235   genotypes, BCC768 developed the lowest PSN in the greenhouse (77±4.5), and

236   BCC192 formed the highest PSN (123±0) in the field (Fig.1B). Interestingly, only two

237   genotypes from the selected six-rowed types produced PSN more than 120 (Fig. 1B),

238   while, in two-rowed, eight genotypes showed PSN greater than or equal to 120

239   (Fig.1A). This indicates that in our selected panel of row-types, more two-rowed

240   genotypes produced higher PSN than six-rowed genotypes. Additionally, the highest

241   PSN among the row-types is also formed by a two-rowed genotype, BCC929 (Fig.

242   1A). Two-rowed genotypes, BCC801, HOR2828, BCC1433, and Hockett, formed

243   significantly less PSN in the field compared to the greenhouse. However, eight of the

244   two-rowed types, BCC1440, BCC929, PI467826, HOR18914, BCC1705, BCC1707,

245   Metcalfe, and Bowman, developed significantly higher PSN in the field. The rest of

246   the two-rowed types showed similar (i.e., statistically non-significant) PSN in the two

247   environments (Fig.1A). Correspondingly, in the six-rowed genotypes, BCC149

248   developed significantly less PSN in the field, while BCC719 and Morex produced

249 more PSN in the field than the greenhouse. The remaining six-rowed genotypes had

250 a similar number of potential spikelets in both environments (Fig.1B). Comparing the

251 mean phenotypic value of the two environments showed that both the row-types

252 have similar mean PSN (Fig. 1C), and they are not significantly different between

253 environments. However, the PSN comparison of every genotype displayed more than

254 half of the genotypes (12 two-rowed and three six-rowed) had a significant difference

255 between the growth conditions (Fig. 1A & B). This result suggested that in barley,

256 PSN is combinatorially influenced by genetic and environmental variations.

257

258 **Survival traits of two-rowed barleys are highly influenced by the environment**

259 The survival traits measure the ability of a plant to produce spikelets (SSL) or grains

260 (GSL). Unlike PSN, SSL and GSL cannot be calculated for every replicate of a

261 genotype because counting the PSN at the MYP stage is a destructive approach.

262 Therefore, it is not possible to relate the spikelet or GN of a replicate to its PSN, and

263 so only the mean values are used for those two traits (Fig 2 & 3). In two-rowed, all

264 genotypes either maintained similar ability or showed a higher level of main culm

265 SSL or GSL in the field (Fig. 2A & 3A). Only one accession, the six-rowed type

266 Morex, reduced its survival traits in the field compared to the greenhouse (Fig. 2B &

267 Fig. 3B). Two-rowed types BCC1440 and BCC1408, had a similar level of SSL, and

268 the rest of the other genotypes had higher SSL in the field (Fig. 2A). Additionally, all

269 the two-rowed genotypes (including BCC1440 and BCC1408) displayed a greater

270 GSL in the field compared to the greenhouse (Fig. 3A). In the six-rowed types,

271 except for Morex, all other genotypes either had similar (BCC768 and BCC766) SSL

272 and GSL in both the environments or had an increasing trend in the field compared to

273 the greenhouse (Fig. 2B & Fig. 3B). In both row-types, the lowest SSL and GSL were

274 formed in the greenhouse and the highest was produced in the field, indicating that

275 field conditions are more amenable for both row-types to exhibit higher survival

276 (spikelet and grain) abilities. The two-rowed genotype BCC801 had the lowest SSL

277 (16.5 %) and GSL (12.6 %), while BCC1433 possessed the highest SSL (28.3 %)

278 and GSL (24.9 %) (Fig. 2A & 3A). Among the six-rowed genotypes, BCC149 had the

279 lowest SSL (45.6 %), while BCC881 had the lowest GSL (26.1 %). The six-rowed

280 genotypes BCC1488 showed the highest SSL (75.2 %), while Newdale had the

281 highest GSL (63 %) (Fig. 2B & Fig. 3B). We did not compare the absolute values of

282 the survival traits between the row-types because two-rowed types, in general, abort

283 two-thirds of their spikelets anyhow (apart from the apical abortion) due to the

284 influence of *Vrs1* gene function (Komatsuda et al., 2007; Sakuma et al., 2013; Zwirek

285 et al., 2018). The average ability of SSL and GSL comparison of the two

286 environments showed that the two-rowed types exhibited higher SSL and GSL in the

287 field, while the six-rowed types only had greater GSL in the field. Additionally, two-

288 rowed types consistently displayed a higher significance ($q<0.001$) for both traits in

289 the field (Fig. 2C & 3C), which indicated that the two-rowed barleys' survival traits

290 (spikelet and grain) are more highly influenced by the environment.

291

292 **Two-rowed barleys exhibited a higher grain set than six-rowed types**

293 Grain set (GS) estimates the potential of a plant to transform the final fertile spikelets

294 into grains successfully. Within our panel, all genotypes either had a similar or higher

295 main culm GS under field conditions (Fig. 4A-C), which reinforced the fact that in the

296 field, our panel of barley row-types performed better than in the greenhouse. The

297 two-rowed genotypes, BCC929, BCC1367, PI467826, BCC1408, BCC1705, and

298 Bowman, as well as the six-rowed types, BCC149, BCC192, BCC881, and BCC161,

299 had a significant increase of GS in the field compared to the greenhouse (Fig. 4A &

300 B). The remaining six- and two-rowed genotypes maintained a similar, i.e., non-

301 significant, GS between the environments. Also, for GS, like SSL and GSL, we

302 observed the lowest GS of both row-types in the greenhouse, while the greatest was

303 in the field. Within two-rowed types, Bowman showed the lowest (66.43±9.07 %) and

304 BCC929, BCC1705, and BCC1707, the highest (~94%) GS (Fig. 4A). Similarly, in the

305 six-rowed types, BCC881 had the lowest (46.91±10.47 %), while Morex and BCC149

306 had the highest (~90%) GS (Fig. 4B). In two-rowed, many genotypes, like BCC1440,

307 BCC929, BCC1367, Garnett, HOR18914, BCC1705, BCC1707, Hockett, and

308 Bowman had greater than or equal to 90% GS in the field compared to two six-rowed

309 genotypes (BCC149 and Morex) (Fig. 4A & B). This suggested that GS of the two-

310 rowed types exhibited a more significant response in the field, which is also reflected

311 in the mean GS comparison between row-types and environments (Fig. 4C). Here,

312 two-rowed types exhibited significantly ($q=0.005$) higher GS ability in the field

313 compared to the greenhouse. Moreover, the two-rowed average GS ability was

314 higher than in the six-rowed types in the greenhouse ($q<0.0001$) and field ($q=0.04$)

315 (Fig. 4C), revealing that two-rowed barleys exhibit the best GS ability after all.

316

317 **Spikelet and grain number are differentially regulated by the environment in**

318 **barley row-types**

319 In our two-rowed panel, except for BCC1408 that had similar SN of the main culm

320 between the environments, all others developed a significantly higher SN in the field

321 (Fig. 5A). Interestingly, all two-rowed genotypes, including BCC1408, formed

322 significantly more grains (main culm) in the field compared to the greenhouse (Fig.

323 6A). Conversely, the six-rowed genotype BCC766 developed significantly fewer

324 spikelets and grains in the field (Fig. 5B & 6B). The remaining six-rowed types either

325 maintained similar or an increased SN (Fig. 5B) and GN (Fig. 6B) in the field

326 compared to the greenhouse. Here also, for both row-types, we found that the lowest

327 SN and GN were in the greenhouse and the highest in the field. In two-rowed, the

328 lowest SN (19.2±1.02) was formed by BCC801 and the highest (35±3.1) by BCC1408

329 (Fig. 5A). Similarly, for GN, the lowest (12.9±2) was produced by Bowman and the

330 highest (32.5±1.8) by BCC929 (Fig. 6A). Comparably, the six-rowed genotypes

331 BCC161 had the lowest (48.1±5.7), and BCC1488 had the highest SN (83.5±3.6)

332 (Fig. 5B). The six-rowed genotype BCC881 formed the lowest GN (31±6.5) and the

333 highest by BCC1488 (66.7±6.1). The average phenotypic value of the SN and GN

334 comparison of the two environments showed that only the two-rowed types

335 developed significantly more SN (Fig. 5C) and GN (Fig. 6C) in the field. In contrast,

336 six-rowed types produced only higher GN in the field. Similar to the findings in SSL,

337 GSL, and GS, we observed that two-rowed types had a consistently higher increase

338 of SN and GN in the field (Fig. 5C & 6C). From this result, it becomes evident that the

339 environment (growth conditions) affects GN of both row-types, but SN only in two-

340 rowed types.

341

342 **Two and six-rowed types have distinct interactions of yield components for**

343 **main culm grain number determination**

344 To reveal the processes influencing main culm GN determination in barley, we

345 assessed the interaction between main culm PSN, SSL, GS, and GN of two- and six-

346 rowed barleys in both environments. In the greenhouse, the GN of the two-rowed

347 was strongly dependent on PSN (R^2=0.68, P<0.0001) and moderately on SSL

348 (R^2=0.35, P=0.012) (Fig. 7A & B). Interestingly, in the field, the interaction between

349 GN and PSN became stronger (R^2=0.86, P<0.0001), while the GN and SSL turned

350 weaker (R^2=0.06, P=0.356) (Fig. 8A & B). In two-rowed, GN seems independent from

12

351   the effect of GS in both environments (Fig. 7C & 8C). Contrastingly under

352   greenhouse conditions, the six-rowed GN was predominantly determined by SSL

353   (R^2=0.56, P=0.012) and GS (R^2=0.49, P=0.025) (Fig. 7E & F). These interactions

354   were significantly reduced in the field. Nevertheless, the SSL (R^2=0.39, P=0.052)

355   ended up as a major yield component, (among other traits) deciding the six-rowed

356   GN (Fig. 8E & F). In both the environments (growth conditions), the six-rowed GN

357   was independent of the influence on PSN (Fig. 7D & 8D). From these results, it is

358   clear that the row-types follow unique yield component interactions for regulating

359   their main culm GN.

360

361   **Discussion**

362   The study was aimed to understand better the interactions between main culm

363   spikelet initiation and growth processes in relation to the main culm GN determination

364   in two- and six-rowed barley. We compared these interactions in two different

365   environments, controlled greenhouse, and field, to assess the stability of their

366   associations. Intriguingly, the PSN was found to be influenced both by the genetical

367   and environmental variations in barley. This observation partly supports the

368   hypothesis that in barley, PSN is a pure developmental factor that is highly controlled

369   by a genetic component, as previously claimed by another study (Arisnabarreta and

370   Miralles, 2006a).

371

372   **Two-rowed barley follows the developmental model for grain number**

373   **determination**

374   In two-rowed, GN was predominantly determined by PSN and to a lesser extent by

375   SSL and GS in both the environments (Fig. 7A & 8A), suggesting that the interaction

376   between PSN and GN is highly stable. Additionally, the above result implied that two-

377   rowed genotypes developed higher PSN at the end of the spikelet initiation phase,

378   produced more grains at harvest compared to the genotypes with fewer PSN,

379   irrespective of their SSL and GS. A similar result was reported in an earlier study

380   (Digel et al., 2015) with a two-rowed barley cv. Scarlett, in which the reduction of the

381   early reproductive phase with a dominant *Ppd-H1* allele significantly diminished the

382   potential spikelets and the number of final floret/spikelet primordia. We also found

383   that the interactions of GN with SN and SN with PSN were robust for two-rowed in

384   both growth conditions (Fig. S1A-D). These interactions reiterated that in two-rowed,

385 GN is mainly determined by the variation in PSN through SN. Field studies on two-

386 and six-rowed barley predicted that the critical period for yield determination could be

387 earlier in two-rowed than six-rowed types (Arisnabarreta and Miralles, 2006b;

388 Arisnabarreta and Miralles, 2008a). Our study also supports this view because we

389 found that the two-rowed GN is predominantly determined by PSN, which is the

390 product of the spikelet initiation phase. Conversely, six-rowed GN had a strong

391 association with SSL, which is a process that happens during the spikelet growth

392 phase, following the spikelet initiation phase. A moderate linear relationship between

393 SSL and GN only in the greenhouse suggested that the two-rowed spikelets

394 competed either with main culm growth or whole plant tiller development during stem

395 elongation, which was not the case in the field (Ghiglione et al., 2008; Gonzalez et

396 al., 2011; Ferrante et al., 2013; Guo and Schnurbusch, 2015; Guo et al., 2017; Guo

397 et al., 2018a; Guo et al., 2018b; Guo et al., 2018c; Perez-Gianmarco et al., 2019).

398 The disconnection of GS with GN in both the environments (Fig. 7C & 8C) suggested

399 that assimilates stored during the pre-anthesis phase were sufficient for grain-filling

400 of two-rowed fertile spikelets. Thus, there is less competition between the spikelets of

401 the two-rowed main culm spike during the GS phase. Collectively, our results

402 supported that the GN determination in two-rowed accessions largely depends on the

403 developmental trait PSN; however, we also found some exceptions in the field. Two-

404 rowed SSL (R^2=0.30, P=0.08) and GS (R^2=0.35, P=0.05) showed a moderate non-

405 linear quadratic (squared) relationship with GN in the field (Fig. S2 A&B), suggesting

406 that the field conditions affected specific two-rowed genotypes that showed either a

407 positive or negative association with GN. Further analysis of these genotypes may

408 reveal more intriguing interactions and influences of SSL and GS to GN. We also

409 verified the interactions in another field study, in which we collected data from

410 random culms instead of from the main culm. Intriguingly, in addition to the strong

411 influence (R^2=0.77, P<0.0001) of PSN on GN, we found that GS had significant

412 (R^2=0.76, P<0.0001) interaction to GN (Fig. S3 A-C). This suggested that in two-

413 rowed different culms might have additional influences in determining GN. Thus, our

414 study exemplified that two-rowed PSN strongly influences GN and that the GN of the

415 two-rowed barley types may follow the developmental model (Bancal, 2008, 2009).

416

417 **Six-rowed grain number follows the survival model**

14

418 In general, the products of the spikelet initiation phase, otherwise known as PSN,

419 enters into the spikelet growth phase during which some potential spikelets or/and

420 spikelet primordia are aborted, most likely and at least partly, due to the competition

421 with the developing main culm and tillers. The end products of the spikelet growth

422 phase are the fertile spikelets (SN) that are filled in the GS phase with assimilates

423 preserved during pre-anthesis. There is also another kind of abortion (known as grain

424 abortion) that happens during the GS phase due to the limited availability and/or

425 allocation of assimilates to the fertile spikelets (Gallagher, 1979). Contrasting to the

426 two-rowed types, six-rowed GN showed a moderate interaction with SSL; but was

427 lowered for PSN both in the greenhouse (Fig. 7D & E) and field (Fig. 8D & E). Also,

428 six-rowed GN exhibited an association with GS only in the greenhouse (Fig 7F).

429 Similar interactions were reported from a field study with the near-isogenic lines of

430 two- and six-rowed barley, demonstrating that the critical period for determining the

431 yield components of six-rowed rather occurred during the survival and part of GS

432 phase (Arisnabarreta and Miralles, 2008a). Also, many studies from wheat claimed

433 that the floret survival period and the competition between florets and other

434 vegetative organs determine final fertile florets (Kirby, 1988; Ghiglione et al., 2008;

435 Gonzalez et al., 2011; Ferrante et al., 2013; Guo and Schnurbusch, 2015; Guo et al.,

436 2017; Guo et al., 2018a; Guo et al., 2018b; Guo et al., 2018c; Perez-Gianmarco et

437 al., 2019). Thus, our results reiterated that the GN of the six-rowed mostly depends

438 on the survival processes irrespective of the growth conditions. It also implied that

439 six-rowed spikelets are highly competitive with other initiation and/or growth events

440 that happen during the spikelet survival phase. The strong interaction of six-rowed

441 SSL with GN indicated that genotypes with high spikelet survival (low spikelet

442 abortion) produce more grains at harvest. The above result is in line with a previous

443 study (Arisnabarreta and Miralles, 2008a), predicting that the critical period for setting

444 grains in six-rowed barley falls within 30 days before heading. A further enhancement

445 of GN potential in six-rowed might be possible if one modulates the survival process

446 (Arisnabarreta and Miralles, 2008a, 2010, 2015), which, however, requires further

447 studies dissecting the genetic modulators of spikelet survival in six-rowed barley. The

448 inconsistent association of GS to GN between the experiments (Fig. 7F & 8F)

449 suggested that the plasticity of GS might be under the environmental influence, which

450 supports the previous outcome observed in a six-rowed field study (Voltas et al.,

451 1997). We only observed an exception in the field conditions, where six-rowed GN

15

452   showed a non-linear quadratic (squared) (R^2=0.73, *P*=0.011) relationship with PSN

453   (Fig. S2C). This indicates that similar to the two-rowed SSL and GS, the field

454   condition impacted certain six-rowed types that showed positive or negative

455   interaction of GN with PSN. We also compared the observations to another field

456   study, in which the random culms of six-rowed types had weak interactions (R^2=0.39,

457   *P*=0.055) between PSN and GN, and the influence of SSL on GN was lost (R^2=0.14,

458   *P*=0.0291). However, GS showed a strong interaction (R^2=0.77, *P*=0.0008) with GN

459   (Fig. S3 D-F). These results suggested that in six-rowed, culms developed in

460   different time windows might have various interactions with GN, as observed in two-

461   rowed barleys. In summary, our results supported that main culm GN determination

462   in six-rowed barley genotypes depends on the SSL; and thus, it rather follows the

463   survival model (Kirby, 1988; Ghiglione et al., 2008; Gonzalez et al., 2011; Ferrante et

464   al., 2013; Perez-Gianmarco et al., 2019).

465

466   **Conclusion**

467   Our study revealed the intrinsic interactions of main culm yield components such as

468   PSN, SSL, and GS with GN of two- and six-rowed barleys in two different growth

469   conditions. We found that GN in two-rowed accessions is majorly determined during

470   the early spikelet initiation phase because it has a strong interaction with PSN in both

471   environments. The spikelets of two-rowed barleys may have moderate or less

472   competition with the other growing parts during the survival phase due to the

473   inconsistent and weak association of SSL with GN. This effect can be partly

474   explained by the two-thirds of the two-rowed spikelets that do not set any grains, i.e.,

475   sterile lateral spikelets. Conversely, in six-rowed, the lateral spikelets form grains, so

476   the GN is mainly determined by the competition of its spikelets within the spike

477   and/or other vegetative/structural organs during the survival phase. A similar

478   hypothesis on the influence of lateral spikelets on two- and six-rowed barley's was

479   predicted earlier (Cottrell and Dale, 1984; Arisnabarreta and Miralles, 2004). Thus,

480   from this study, we therefore, propose that the barley row-types follow two distinct

481   patterns of GN determination, where two-rowed barleys follow a developmental

482   model, and six-rowed genotypes comply with the survival model.

483

484   **Supplementary data**

16

485   Figure S1. Interactions of two-rowed potential spikelet number, final spikelet number,

486   and grain number in greenhouse and field.

487   Figure S2. Interactions of potential spikelet number, spikelet survival, grain set, and

488   grain number in greenhouse and field.

489   Figure S3: Interactions of random culms' potential spikelet number, spikelet survival,

490   grain set, and grain number in the field.

491   Table S1: Details of the 27 accessions used in this study.

492   Table S2: Growth conditions of the field and greenhouse experiments.

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494

495   **Acknowledgments**

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505   **Author contributions**

506   T.S. and V.T. conceived the idea for this experiment; V.T., and R.K., conducted the

507   experiments; V.T. analyzed the data and wrote the manuscript; T.S. edited and

508   reviewed the manuscript together with R.K.; T.S. supervised the study.

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714

715

716   **Figure legends:**

717   Figure 1. **Potential spikelet number variation of row-types in the greenhouse**

718   **and field.** A comparison of the main culm potential spikelet number (PSN) between

719   greenhouse and field conditions of two-rowed (A) and six-rowed barleys (B) is shown.

720   A) Two-rowed genotypes BCC801, HOR2828, BCC1433, and Hockett showed a

721   reduction, whereas BCC1440, BCC929, PI467826, HOR18914, BCC1705,

722   BCC1707, Metcalfe, and Bowman exhibited an increase in PSN in the field. B) In six-

723   rowed, only BCC149 developed a reduced PSN in the field compared to the

724   greenhouse. Contrarily, genotypes BCC719 and Morex formed more PSN in the field.

725   C) A comparison of the mean values of the PSN between greenhouse and field of

726   two- and six-rowed genotypes is shown. PSN is not significantly different between

727   row-types and the environments. In A & B, each genotype was represented by three

728   replicates both in the greenhouse and field. In C, the mean values of 17 two-rowed

729   and ten six-rowed genotypes were used. Data in A & B were analyzed by *multiple*

730   *Student's t-tests* with false discovery analysis of *Benjamini, Kreier, and Yekutieli* with

731   the *q* value of 5% and the data in C with *Two-way ANOVA* with *Tukey's multiple*

732   *comparison test (alpha=5%)*; Letter 'a' in panel C denotes that the mean values are

733   not significantly different at $q>0.05$. *, $q≤0.05$; **, $q≤0.01$; ***, $q≤0.001$; ns, *q>0.05*.

734

735   Figure 2. **Spikelet survival variation of row-types in the greenhouse and field.** A

736   comparison of the mean main culm spikelet survival (SSL) between the greenhouse

737   and field conditions of two-rowed (A) and six-rowed barleys (B) is shown. A) In two-

738   rowed, except BCC1440 and BCC1408, all other genotypes exhibited a higher trend

739   of SSL in the field. B) In six-rowed, genotypes BCC149, BCC192, BCC881, BCC161,

740   BCC719, BCC1488, and Newdale showed a trend of increased SSL in the field

741   compared to the greenhouse while Morex had a decreased trend in the field. C) A

742   comparison of the mean values of SSL between the greenhouse and field of two- and

743   six-rowed genotypes is shown. Two-rowed showed an increased variation of SSL in

744   the field, while six-rowed exhibited similar variation between the environments. In A &

745   B, each genotype was represented by the mean value from 4 to 12 replicates in the

746   greenhouse and six replicates in the field. In C, the mean values of 17 two-rowed and

747   10 six-rowed genotypes were used. In C, the data were analyzed by *paired Student's*

748   *t-test (parametric)* with false discovery analysis of *Benjamini, Kreier, and Yekutieli*

749   with the *q* value of 5%; ***, $q≤0.001$; ns, *q>0.05*.

23

750   Figure 3. **Grain survival variation of row-types in the greenhouse and field.** A
751   comparison of the mean main culm grain survival (GSL) between the greenhouse
752   and field conditions of two-rowed (A) and six-rowed barleys (B) is shown. A) In two-
753   rowed, all genotypes exhibited a higher trend of grain survival in the field. B) In six-
754   rowed, except Morex that showed a reduced SSL and GSL in the field, the rest of
755   other genotypes either had similar (BCC768 and BCC766) GSL in both the
756   environments or had an increasing trend of GSL in the field compared to the
757   greenhouse. C) A comparison of the mean values of GSL between greenhouse and
758   field of two- and six-rowed genotypes is shown. Both row-types showed increased
759   GSL in the field, while two-rowed genotypes showed a more significant increase than
760   six-rowed. In A & B, each genotype was represented by the mean value from 4 to 12
761   replicates in the greenhouse and six replicates in the field. In C, the mean values of
762   17 two- and ten six-rowed genotypes were used. In C, the data were analyzed by
763   *paired Student's t-test (parametric)* with false discovery analysis of *Benjamini, Kreier,*
764   *and Yekutieli* with the q value of 5%; ***, $q≤0.001$; *, *$q≤0.05$*.

765

766   Figure 4. **Grain set variation of row-types in the greenhouse and field.** A
767   comparison of the main culm grain set (GS) between greenhouse and field conditions
768   of two-rowed (A) and six-rowed barleys (B) is shown. A) In two-rowed, genotypes
769   BCC929, BCC1367, PI467826, BCC1408, BCC1705, and Bowman showed higher
770   GS in the field. B) In six-rowed, genotypes BCC149, BCC192, BCC881, and
771   BCC161 had significantly higher GS in the field compared to the greenhouse. C) A
772   comparison of the mean values of GS between the greenhouse and field of the two-
773   and six-rowed genotypes is shown. Two-rowed showed an increased variation of GS
774   in the field, while six-rowed exhibited similar variation between the environments. In A
775   & B, each genotype was represented by 4 to 12 replicates in the greenhouse and six
776   replicates in the field. In C, the mean values of 17 two- and ten six-rowed genotypes
777   were used. Data in A & B were analyzed by *multiple Student's t-tests* with false
778   discovery analysis of *Benjamini, Kreier, and Yekutieli* with the q value of 5% and the
779   data in C with *Two-way ANOVA* with *Tukey's multiple comparison test (alpha=5%)*;
780   Letter 'b' in panel C shows that the two-rowed field GS is significantly different than
781   two-rowed greenhouse ($q≤0.001$), six-rowed greenhouse ($q≤0.001$), and six-rowed
782   field ($q≤0.05$). However, the letter 'a' denotes that the mean values are not
783   significantly different at $q>0.05$. *, $q≤0.05$; **, $q≤0.01$; ***, $q≤0.001$; ns, *$q>0.05$*.

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784 Figure 5. **Final spikelet number variation of row-types in the greenhouse and**

785 **field.** A comparison of the main culm spikelet number (SN) between greenhouse and

786 field conditions of two-rowed (A) and six-rowed barleys (B) is shown. A) Except for

787 the two-rowed type BCC1408 that have similar SN between the environments, all

788 others were developed a significantly higher SN in the field. B) In six-rowed, BCC766

789 developed significantly less SN in the field. At the same time, the rest of the other

790 genotypes either maintained similar (BCC768, BCC881, & Morex) or produced

791 significantly higher (BCC149, BCC192, BCC161, BCC719, BCC1488, and Newdale)

792 SN in the field. C) A comparison of the mean values of the SN between the

793 greenhouse and field of two- and six-rowed genotypes is shown. Two-rowed showed

794 significantly higher variation in the field while six-rowed had similar variation in both

795 growth conditions. In A & B, each genotype was represented by 4 to 12 replicates in

796 the greenhouse and six replicates in the field. In C, the mean values of 17 two- and

797 ten six-rowed genotypes were used. Data in A &B were analyzed by *multiple*

798 *Student's t-tests* and the data in C with *paired Student's t-test* (*parametric*) with false

799 discovery analysis of *Benjamini, Kreier, and Yekutieli* with the *q* value of 5%; *,

800 *q*≤0.05; **, *q*≤0.01; ***, *q*≤0.001; ns, *q>0.05*.

801

802 Figure 6. **Grain number variation of row-types in the greenhouse and field.** A

803 comparison of the main culm grain number (GN) between greenhouse and field

804 conditions of two-rowed (A) and six-rowed barleys (B) is shown. A) All two-rowed

805 genotypes formed a higher GN significantly in the field compared to the greenhouse.

806 B) The six-rowed genotype BCC766 produced significantly lower GN in the field while

807 the rest of the other genotypes either maintained similar (BCC768, Morex) or higher

808 GN in the field. C) A comparison of the mean values of GN between greenhouse and

809 field of two- and six-rowed genotypes is shown. Both row-types developed

810 significantly more grains in the field. In A & B, each genotype was represented by 4

811 to 12 replicates in the greenhouse and six replicates in the field. In C, the mean

812 values of 17 two- and ten six-rowed genotypes were used. Data in A &B were

813 analyzed by *multiple Student's t-tests* and the data in C with *paired Student's t-test*

814 (*parametric*) with false discovery analysis of *Benjamini, Kreier, and Yekutieli* with the

815 *q* value of 5%; *, *q*≤0.05; **, *q*≤0.01; ***, *q*≤0.001; ns, *q>0.05*.

816

817 Figure 7. **Interactions of potential spikelet number, spikelet survival, and grain**

818 **set with grain number in the greenhouse.** The interactions of potential spikelet

819 number (PSN) with grain number (GN), spikelet survival (SSL) with GN, and grain set

820 (GS) with GN of two- (A, B, & C) and six-rowed (D, E, & F) are shown respectively. In

821 two-rowed, GN was predominantly determined by PSN, while in six-rowed by SSL

822 and GS. Linear regression analysis was done by considering GN as dependent and

823 PSN or SSL or GS as independent traits for both row-types. 95% confidence intervals

824 were plotted as confidence bands.

825

826 Figure 8. **Interactions of potential spikelet number, spikelet survival, and grain**

827 **set with grain number in the field.** The interactions of potential spikelet number

828 (PSN) with grain number (GN), spikelet survival (SSL) with GN, and grain set (GS)

829 with GN of two- (A, B, & C) and six-rowed (D, E, & F) are shown respectively. In two-

830 rowed, GN was predominantly determined by PSN, while in six-rowed by SSL. Linear

831 regression analysis was done by considering GN as dependent and PSN or SSL or

832 GS as independent traits for both row-types. A 95% confidence interval was plotted

833 as confidence bands.

834

835 **Supplementary figure legends:**

836 Figure S1. **Interactions of two-rowed potential spikelet number, final spikelet**

837 **number, and grain number in greenhouse and field.** The interaction of the main

838 culm final spikelet number (SN) with grain number (GN) of two-rowed barleys in the

839 greenhouse (A) and field (C) is shown. Similarly, the interaction of potential spikelet

840 number (PSN) with final SN of two-rowed barleys in the greenhouse (B) and field (D)

841 is shown. Linear regression analysis was done by considering either GN (A & C) or

842 SN (B & D) as dependent and SN (A & C) or PSN (B & D) as independent traits for

843 two-rowed types. 95% confidence intervals were plotted as confidence bands.

844

845 Figure S2. **Interactions of potential spikelet number, spikelet survival, grain set,**

846 **and grain number in greenhouse and field.** The non-linear interaction of main culm

847 spikelet survival (SSL) with grain number (GN) and main culm grain set (GS) with GN

848 of two-rowed barleys in field conditions shown in A & B respectively. In C, the non-

849 linear interaction of six-rowed main culm potential spikelet number (PSN) with GN in

850 the field condition is shown. In A, B & C, we found a non-linear quadratic (squared)

26

851 relationship with respected dependent and independent traits. The analysis was done

852 by considering GN as dependent and SSL (A) or GS (B) or PSN (C) as independent

853 traits.

854

855 Figure S3: **Interactions of random culms' potential spikelet number, spikelet**

856 **survival, grain set, and grain number in the field.** The interactions of potential

857 spikelet number (PSN) with grain number (GN), spikelet survival (SSL) with GN, and

858 grain set (GS) with GN of two- (A, B, & C) and six-rowed (D, E, & F) are shown

859 respectively. A 95% confidence interval was plotted as confidence bands.
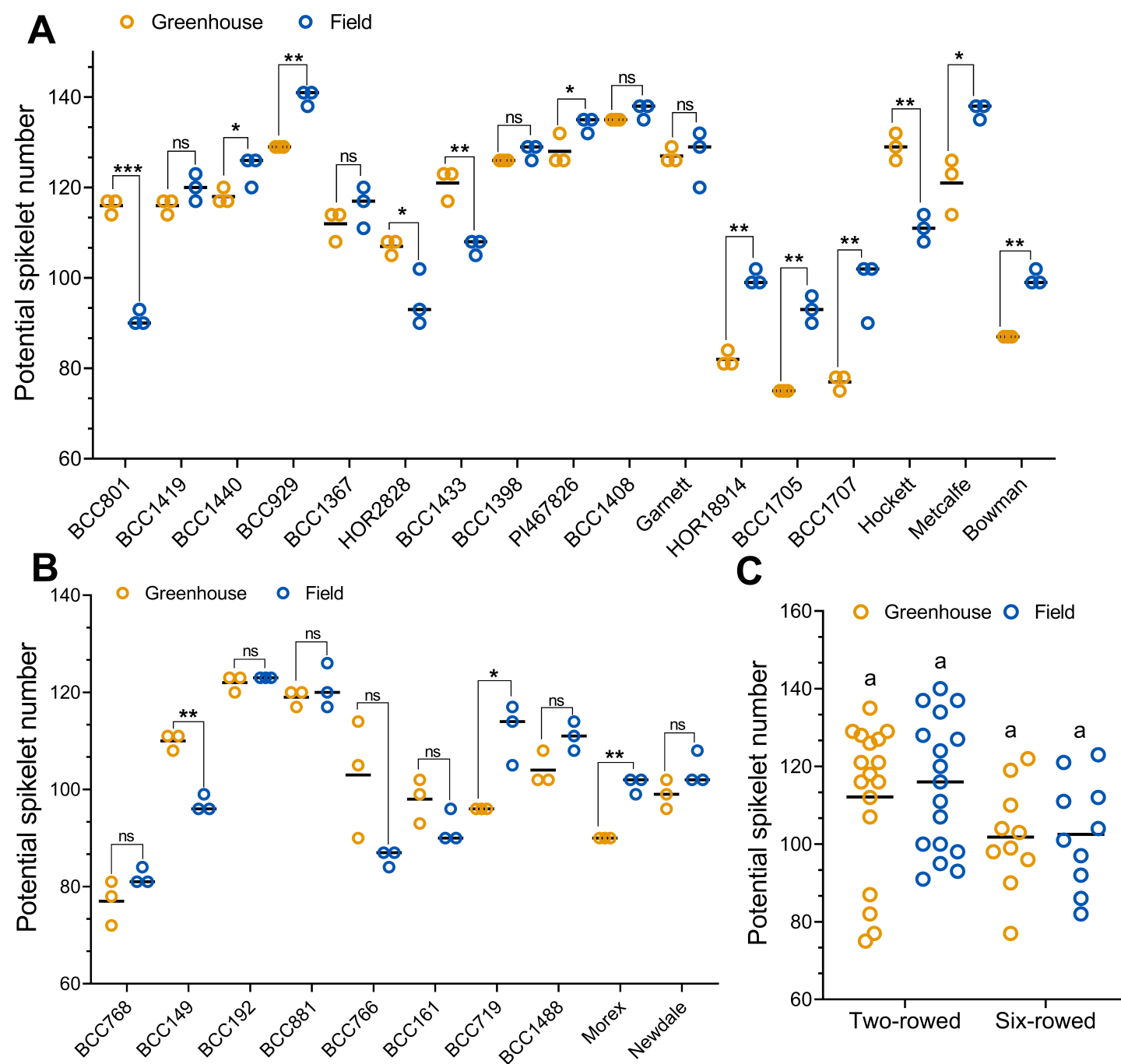
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Figure 1. Potential spikelet number variation of row-types in the greenhouse and field. A comparison of the main culm potential spikelet number (PSN) between greenhouse and field conditions of two-rowed (A) and six-rowed barleys (B) is shown. A) Two-rowed genotypes BCC801, HOR2828, BCC1433, and Hockett showed a reduction, whereas BCC1440, BCC929, PI467826, HOR18914, BCC1705, BCC1707, Metcalfe, and Bowman exhibited an increase in PSN in the field. B) In six-rowed, only BCC149 developed a reduced PSN in the field compared to the greenhouse. Contrarily, genotypes BCC719 and Morex formed more PSN in the field. C) A comparison of the mean values of the PSN between greenhouse and field of two- and six-rowed genotypes is shown. PSN is not significantly different between row-types and the environments. In A & B, each genotype was represented by three replicates both in the greenhouse and field. In C, the mean values of 17 two-rowed and ten six-rowed genotypes were used. Data in A & B were analyzed by multiple Student's t-tests with false discovery analysis of Benjamini, Kreier, and Yekutieli with the q value of 5% and the data in C with Two-way ANOVA with Tukey's multiple comparison test (alpha=5%); Letter 'a' in panel C denotes that the mean values are not significantly different at q>0.05. *, q≤0.05; **, q≤0.01; ***, q≤0.001; ns, q>0.05.
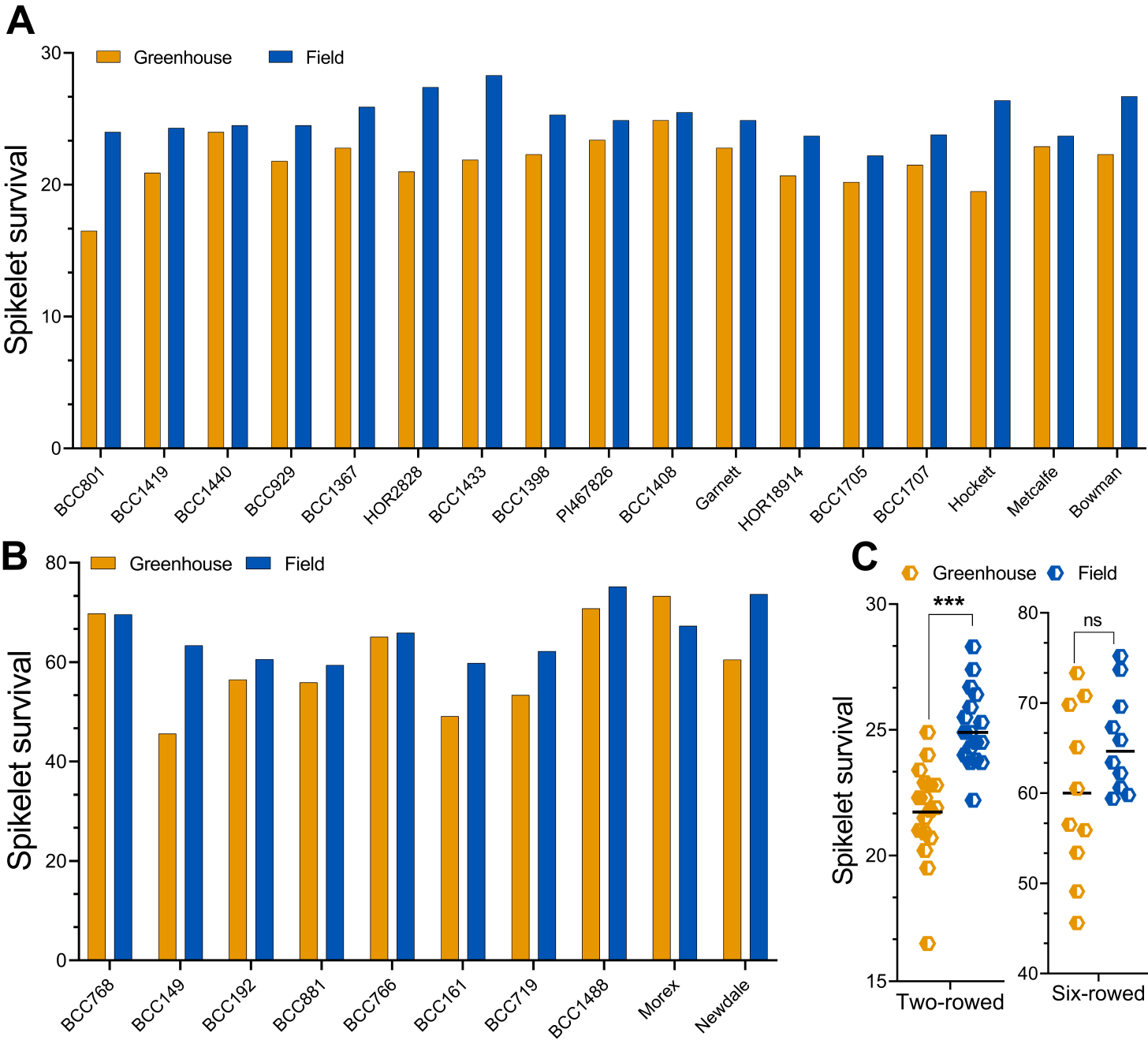
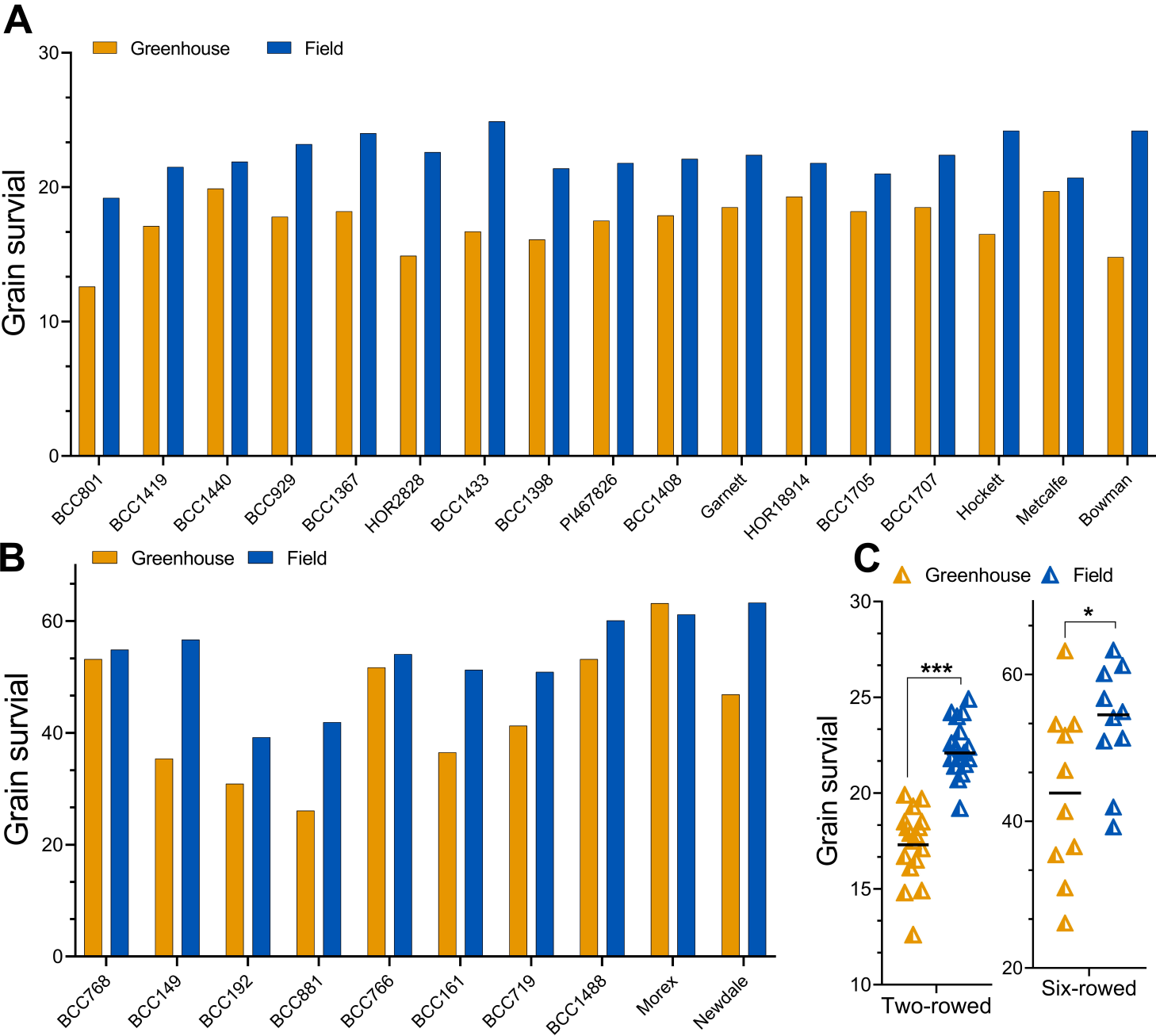Figure 2. Spikelet survival variation of row-types in the greenhouse and field. A comparison of the mean main culm spikelet survival (SSL) between the greenhouse and field conditions of two-rowed (A) and six-rowed barleys (B) is shown. A) In two-rowed, except BCC1440 and BCC1408, all other genotypes exhibited a higher trend of SSL in the field. B) In six-rowed, genotypes BCC149, BCC192, BCC881, BCC161, BCC719, BCC1488, and Newdale showed a trend of increased SSL in the field compared to the greenhouse while Morex had a decreased trend in the field. C) A comparison of the mean values of SSL between the greenhouse and field of two- and six-rowed genotypes is shown. Two-rowed showed an increased variation of SSL in the field, while six-rowed exhibited similar variation between the environments. In A & B, each genotype was represented by the mean value from 4 to 12 replicates in the greenhouse and six replicates in the field. In C, the mean values of 17 two-rowed and 10 six-rowed genotypes were used. In C, the data were analyzed by paired Student's t-test (parametric) with false discovery analysis of Benjamini, Kreier, and Yekutieli with the q value of 5%; ***, q≤0.001; ns, q>0.05.

Figure 3. Grain survival variation of row-types in the greenhouse and field. A comparison of the mean main culm grain survival (GSL) between the greenhouse and field conditions of two-rowed (A) and six-rowed barleys (B) is shown. A) In two-rowed, all genotypes exhibited a higher trend of grain survival in the field. B) In six-rowed, except Morex that showed a reduced SSL and GSL in the field, the rest of other genotypes either had similar (BCC768 and BCC766) GSL in both the environments or had an increasing trend of GSL in the field compared to the greenhouse. C) A comparison of the mean values of GSL between greenhouse and field of two- and six-rowed genotypes is shown. Both row-types showed increased GSL in the field, while two-rowed genotypes showed a more significant increase than six-rowed. In A & B, each genotype was represented by the mean value from 4 to 12 replicates in the greenhouse and six replicates in the field. In C, the mean values of 17 two- and ten six-rowed genotypes were used. In C, the data were analyzed by paired Student's t-test (parametric) with false discovery analysis of Benjamini, Kreier, and Yekutieli with the q value of 5%; ***, $q \leq 0.001$; *, $q \leq 0.05$.
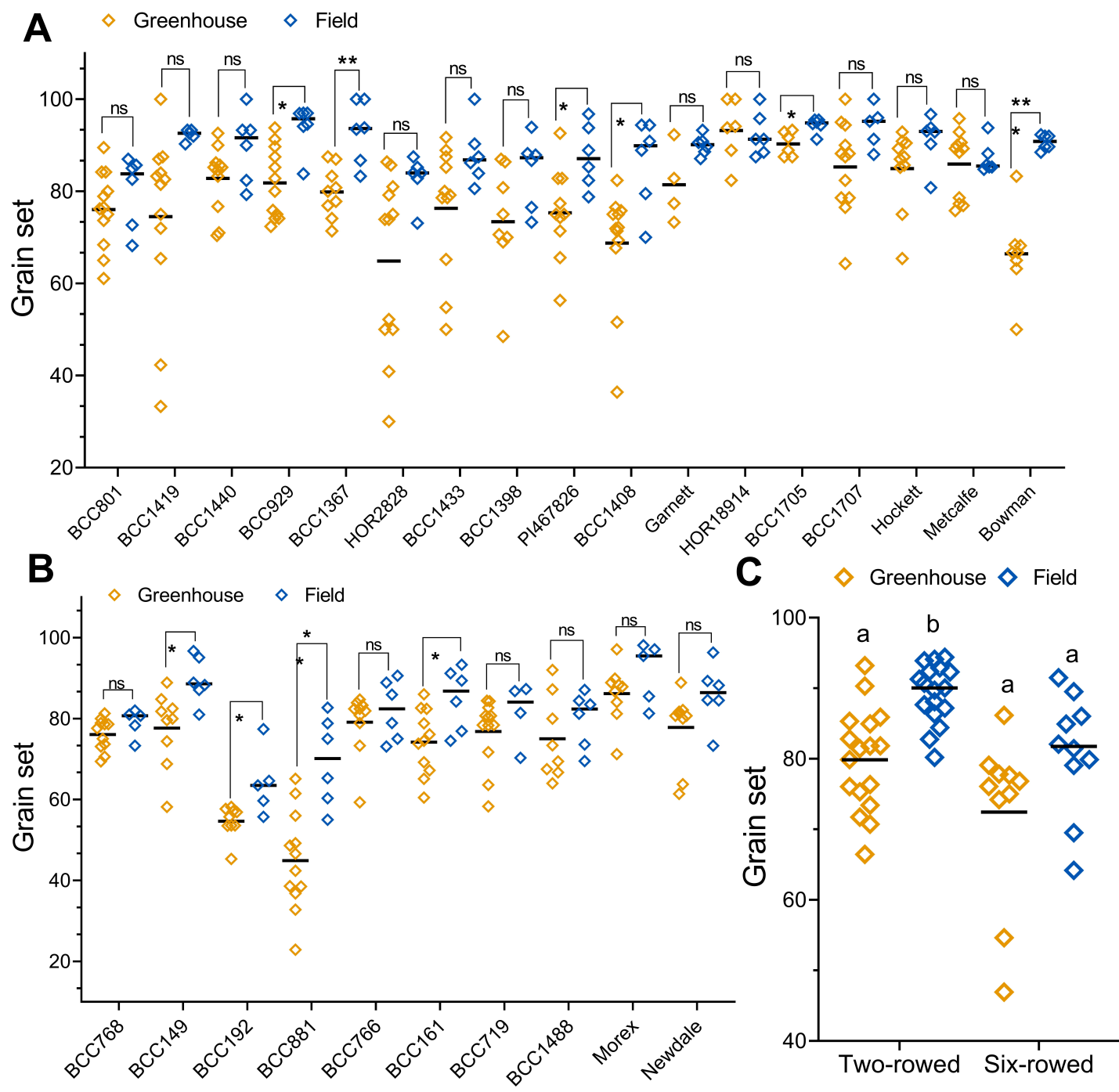
Figure 4

Figure 4. Grain set variation of row-types in the greenhouse and field. A comparison of the main culm grain set (GS) between greenhouse and field conditions of two-rowed (A) and six-rowed barleys (B) is shown. A) In two-rowed, genotypes BCC929, BCC1367, PI467826, BCC1408, BCC1705, and Bowman showed higher GS in the field. B) In six-rowed, genotypes BCC149, BCC192, BCC881, and BCC161 had significantly higher GS in the field compared to the greenhouse. C) A comparison of the mean values of GS between the greenhouse and field of the two- and six-rowed genotypes is shown. Two-rowed showed an increased variation of GS in the field, while six-rowed exhibited similar variation between the environments. In A & B, each genotype was represented by 4 to 12 replicates in the greenhouse and six replicates in the field. In C, the mean values of 17 two- and ten six-rowed genotypes were used. Data in A & B were analyzed by multiple Student's t-tests with false discovery analysis of Benjamini, Kreier, and Yekutieli with the q value of 5% and the data in C with Two-way ANOVA with Tukey's multiple comparison test (alpha=5%); Letter 'b' in panel C shows that the two-rowed field GS is significantly different than two-rowed greenhouse (q≤0.001), six-rowed greenhouse (q≤0.001), and six-rowed field (q≤0.05). However, the letter 'a' denotes that the mean values are not significantly different at q>0.05. *, q≤0.05; **, q≤0.01; ***, q≤0.001; ns, q>0.05.
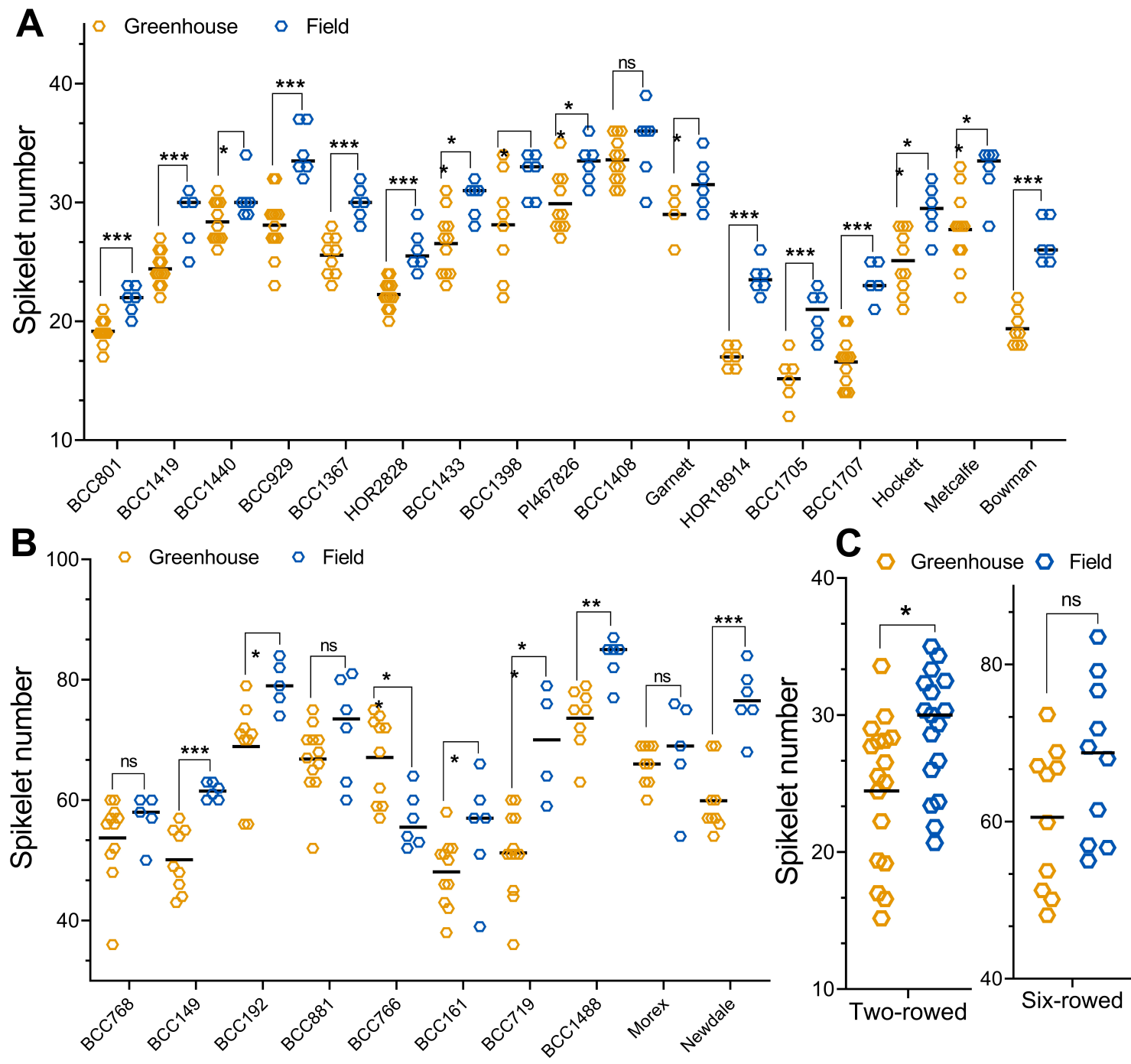
Figure 5. Final spikelet number variation of row-types in the greenhouse and field. A comparison of the main culm spikelet number (SN) between greenhouse and field conditions of two-rowed (A) and six-rowed barleys (B) is shown. A) Except for the two-rowed type BCC1408 that have similar SN between the environments, all others were developed a significantly higher SN in the field. B) In six-rowed, BCC766 developed significantly less SN in the field. At the same time, the rest of the other genotypes either maintained similar (BCC768, BCC881, & Morex) or produced significantly higher (BCC149, BCC192, BCC161, BCC719, BCC1488, and Newdale) SN in the field. C) A comparison of the mean values of the SN between the greenhouse and field of two- and six-rowed genotypes is shown. Two-rowed showed significantly higher variation in the field while six-rowed had similar variation in both growth conditions. In A & B, each genotype was represented by 4 to 12 replicates in the greenhouse and six replicates in the field. In C, the mean values of 17 two- and ten six-rowed genotypes were used. Data in A &B were analyzed by multiple Student's t-tests and the data in C with paired Student's t-test (parametric) with false discovery analysis of Benjamini, Kreier, and Yekutieli with the q value of 5%; *, q≤0.05; **, q≤0.01; ***, q≤0.001; ns, q>0.05.
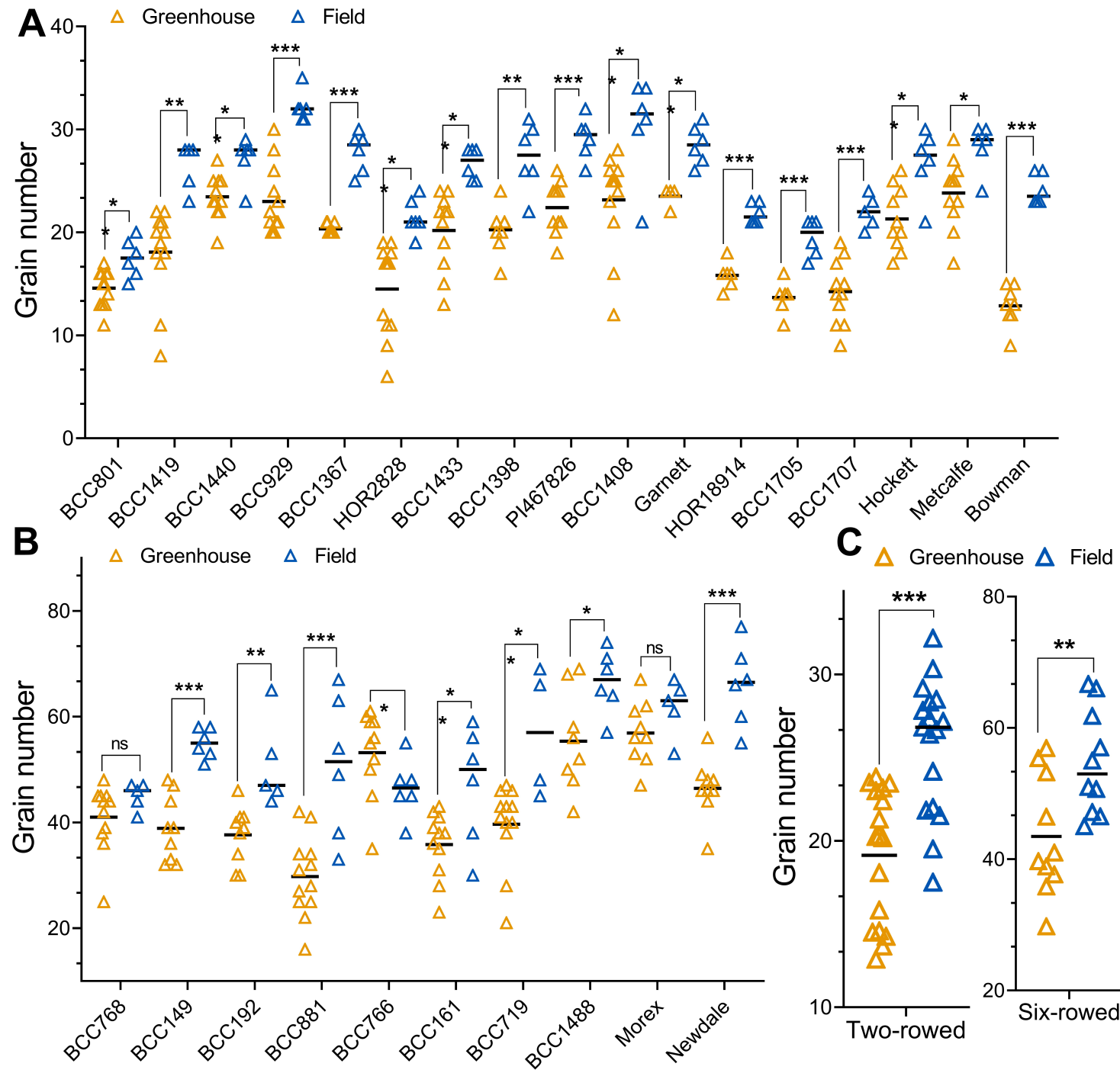
Figure 6. Grain number variation of row-types in the greenhouse and field. A comparison of the main culm grain number (GN) between greenhouse and field conditions of two-rowed (A) and six-rowed barleys (B) is shown. A) All two-rowed genotypes formed a higher GN significantly in the field compared to the greenhouse. B) The six-rowed genotype BCC766 produced significantly lower GN in the field while the rest of the other genotypes either maintained similar (BCC768, Morex) or higher GN in the field. C) A comparison of the mean values of GN between greenhouse and field of two- and six-rowed genotypes is shown. Both row-types developed significantly more grains in the field. In A & B, each genotype was represented by 4 to 12 replicates in the greenhouse and six replicates in the field. In C, the mean values of 17 two- and ten six-rowed genotypes were used. Data in A &B were analyzed by multiple Student's t-tests and the data in C with paired Student's t-test (parametric) with false discovery analysis of Benjamini, Kreier, and Yekutieli with the q value of 5%; *, q≤0.05; **, q≤0.01; ***, q≤0.001; ns, q>0.05.
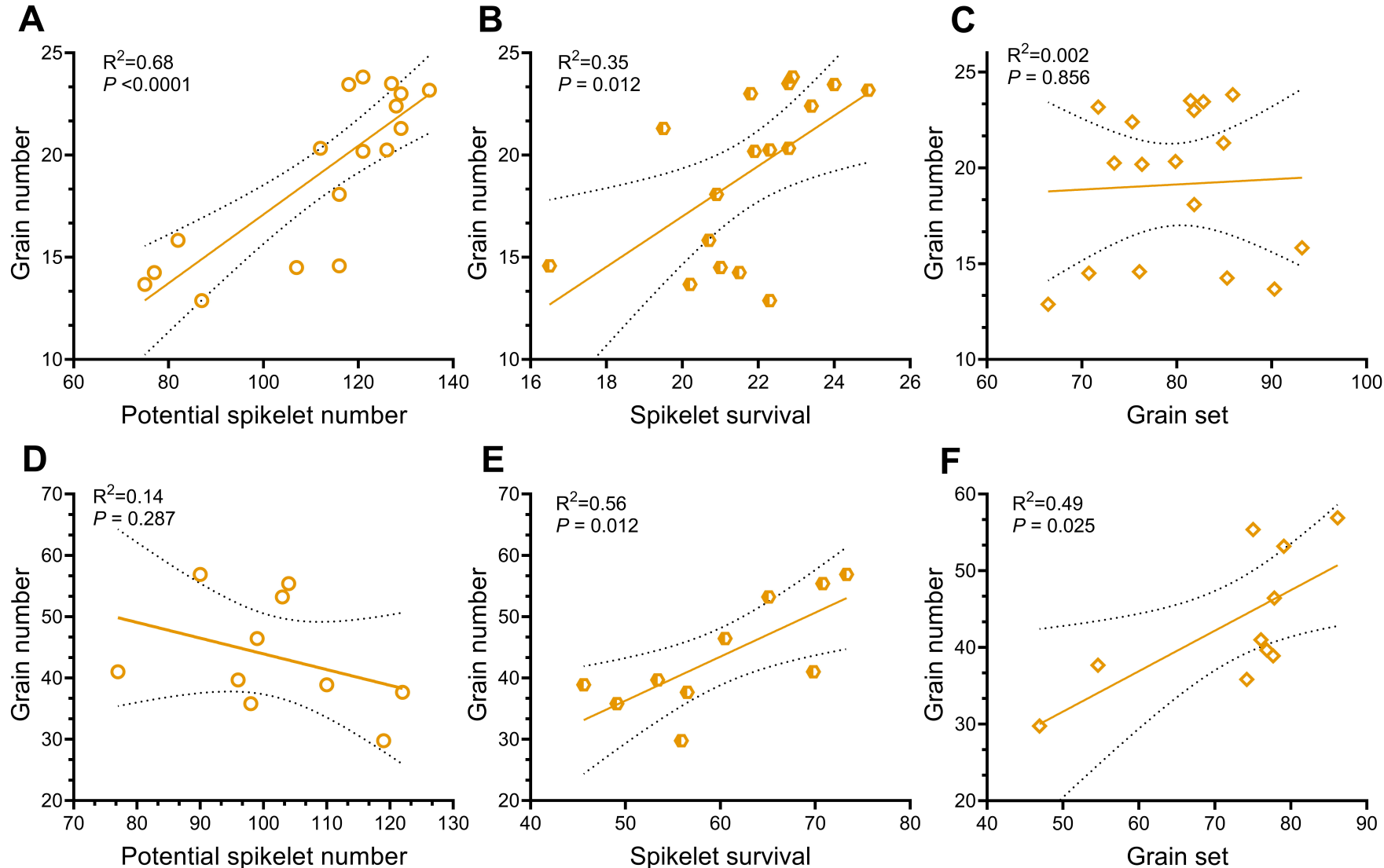
Figure 7. Interactions of potential spikelet number, spikelet survival, and grain set with grain number in the greenhouse. The interactions of potential spikelet number (PSN) with grain number (GN), spikelet survival (SSL) with GN, and grain set (GS) with GN of two- (A, B, & C) and six-rowed (D, E, & F) are shown respectively. In two-rowed, GN was predominantly determined by PSN, while in six-rowed by SSL and GS. Linear regression analysis was done by considering GN as dependent and PSN or SSL or GS as independent traits for both row-types. 95% confidence intervals were plotted as confidence bands.
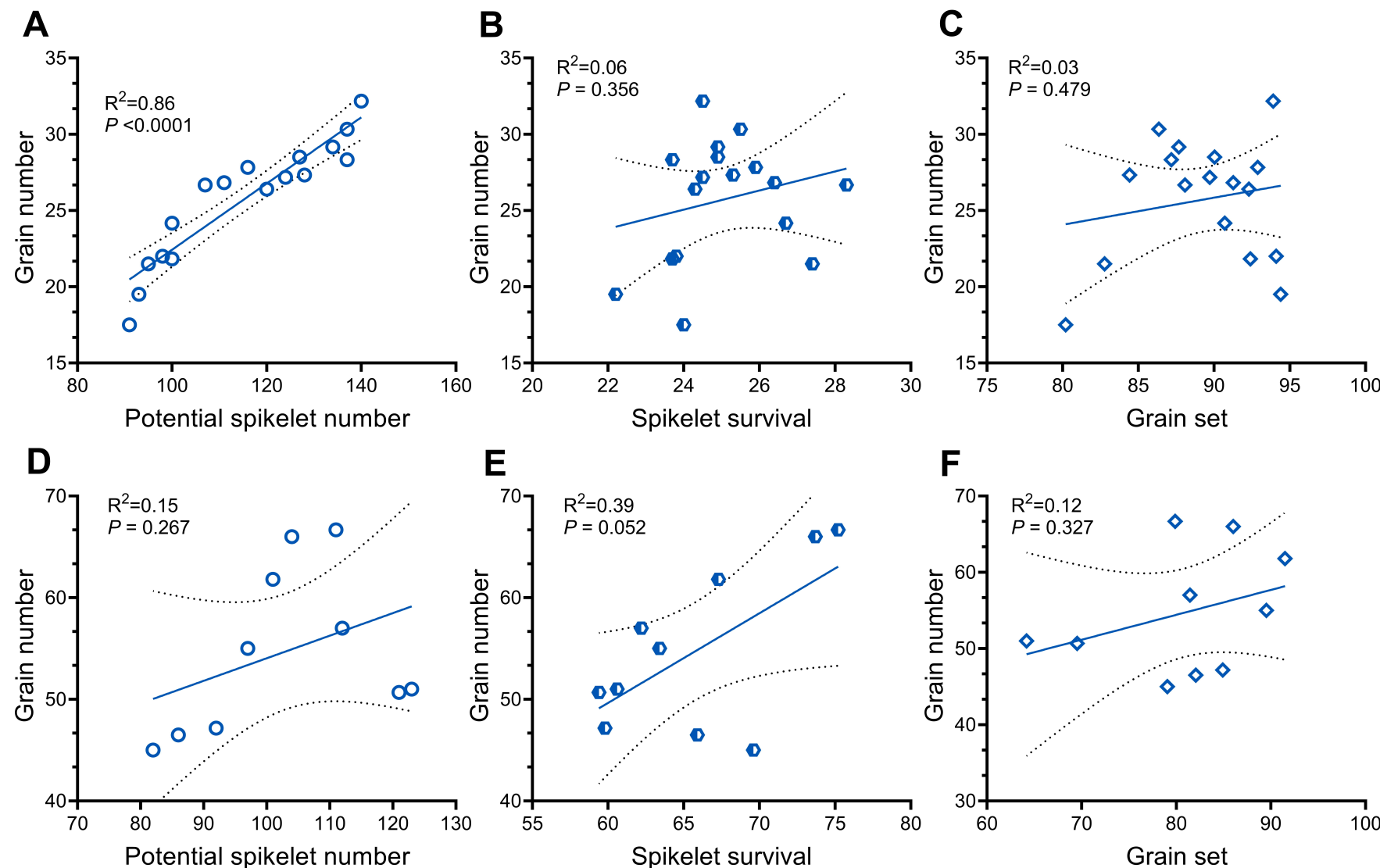
Figure 8. Interactions of potential spikelet number, spikelet survival, and grain set with grain number in the field. The interactions of potential spikelet number (PSN) with grain number (GN), spikelet survival (SSL) with GN, and grain set (GS) with GN of two- (A, B, & C) and six-rowed (D, E, & F) are shown respectively. In two-rowed, GN was predominantly determined by PSN, while in six-rowed by SSL. Linear regression analysis was done by considering GN as dependent and PSN or SSL or GS as independent traits for both row-types. A 95% confidence interval was plotted as confidence bands.