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Article

Density and population arrangement of off-season corn cultivars: agronomic parameters and leaf burn by frost

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ABSTRACT: Off-season corn is the main form of corn crop in Brazil. During the maturation stage of the plants, the availability of heat is reduced, and frosts may occur. The aim of this study was to determine the effect of row spacing reduction and population increase in corn cultivars on agronomic parameters and leaf burn caused by moderate frost to minimize the effects of abiotic stresses on grain yield. We used a randomized block experimental design in a 3 × 4 × 2 factorial scheme: row spacing of 40, 80, and 120 cm; populations of 25,000, 43,750, 62,500, and 81,250 plants·ha^{-1}; and Fort cultivars (semi-erect leaves, and early cycle) and DKB 950 (erect leaves and super-early cycle). Three field trials were conducted in the state of São Paulo, Brazil, from mid-March. The spacing reduction provided antagonistic effects on yield with different response from hybrids in the most of locations. Population density increased plant height, stalk lodging and grain yield; populations ≥ 68,000 plants per hectare provided the best yields. Specific determinations were made in the area where moderate frost occurred; higher leaf area index and less Brix in the stem at 40 cm spacing were obtained, but without any increase in plant dry mass and grain yield. The hybrid with erect leaves was more adapted to the reduced spacing and exhibited less leaf damage by frost. The effect of frost was greater at reduced row spacings; therefore, this factor should be considered when selecting the population arrangement of off-season corn in areas with frequent frost.

Key words: reduced spacing, leaf area index, dry mass, grain yield.

INTRODUCTION

The production of off-season corn or second crop without irrigation is the main type of cultivation of this cereal in Brazil, accounting for three-quarter of the area and national production (CONAB 2022). The corn is sown from January to March, and during its development, in autumn and winter, environmental conditions become more unfavorable, with reduction in day length, temperature, and rainfall. In the Vale do Paranapanema region, in the state of São Paulo, Brazil, the thermal factor is a major limitation, and moderate and severe frosts may occur in one-third and one-quarter of the year, respectively (Pantano 2017).

The population of plants used in off-season corn production is relatively low, usually 55 to 65 thousand plants per hectare, and the yields in the range of 6–9 tons per hectare (Duarte et al. 2017). The use of 80 and 90 cm spacing has reduced markedly, and a reduced spacing of 45 and 50 cm has become predominant during the 2010s (Duarte 2022), mainly due to the operational ease of using the same planter and spacing for growing soybeans.

Corn grain yield was increased through the development of cultivars that were more adapted to high population density. These cultivars had a lower rate of plant barrenness and stalk lodging in high-density field crops (Duvick 2005, Ma et al. 2014) and less competition between plants for solar radiation due to more erect leaves (Strieder et al. 2007). Densification enables greater accumulation of dry mass per area and greater leaf area index, which stabilize after a certain threshold

(Ahmad et al. 2010, Overman and Scholtz 2011, Djaman et al. 2022, Graffitti et al. 2021). However, yield may be reduced in extremely high-density plantations (Zhai et al. 2017, Bernhard and Below 2020).

The spatial distribution of plants can also interfere with maize yield. Reduction in spacing generally increases yield because more equidistant distribution of plants maximizes the use of light, water, and nutrients (Sangoi et al. 2001). However, there are interactions between spacing, plant population, and cultivars, mainly due to differences in plant cycle and architecture (Strieder et al. 2007, Ahmad et al. 2010, Kappes et al. 2011, Djaman et al. 2022). The beneficial effects of reduced spacing have often been associated with high populations and vice versa (Cruz et al. 2007, Bernhard and Below 2020).

Studies on population arrangement in off-season corn are few. Dias et al. (2019) reported that reduction in row spacing did not influence plant architecture, grain yield, and its components, regardless of population and cultivar. The same found Schwantes et al. (2007), that observed that yield did not differ between width rows of 0.36 and 0.78 m. However, Penariol et al. (2003) showed that reduction in row spacing to 40 cm increased yield and reduced plant heights in the contrasting genotypes in terms of cycle and plant architecture.

Information on cold tolerance in corn grown during the winter season in a tropical/subtropical environment is limited (Zaidi et al. 2010). Corn does not tolerate temperatures below 0 °C, unlike temperate plants, which reduce their rate of development and become more tolerant to subsequent exposure to cooling temperatures through progressive changes in cell structure and metabolism (Harwood 1991). Low photosynthetic performance and export of assimilates from corn leaves occur at low temperatures due to changes in the ultrastructure of plasmodesmata (Bilska and Sowinski 2010) and disturbances in chloroplast development, changes to the pigment composition, damage of the photosystem II (PSII) reaction centers, and reduced activity of carbon cycle enzymes (Zhou et al. 2022). Below a certain temperature threshold or severe frost, ice forms in the cells of maize and damages them permanently. The ice crystals in the intercellular space break down chloroplast structures and cause a rapid change in leaf biochemical constituents (Choudhury et al. 2019).

Given that temperatures gradually decrease during autumn–winter and that studies on the interaction between density, population arrangement, and genotypes of off-season corn under these conditions are scarce in the São Paulo region of Médio Paranapanema, especially with the occurrence of frost, the present study was conducted with the aim of identifying crop managements that minimize the effects of abiotic stresses on grain yield.

MATERIALS AND METHODS

The experiments were conducted under field conditions and no-tillage in a Dystroferric Red Latosol in the municipalities of Cândido Mota, Pedrinhas Paulista and Palmital, state of São Paulo.

A randomized block design was used with four replicates in a 3 × 4 × 2 factorial scheme. Three row spacings were used (40, 80, and 120 cm), in four plant populations (25,000, 43,750, 62,500, and 81,250 plants·ha^{-1}) and two cultivars: Fort (Syngenta single hybrid, tall, semi-erect leaves, and early cycle) and DKB 950 (Dekalb's single hybrid, low height, erect leaves, and super early cycle), that represent the types of architecture and cycle of plants cultivated in this region.

The plots were 7-m long and had four, five, and 10 rows with 120, 80 and 40 cm spacing, respectively. A total of 5 m of the two central rows were used for evaluations at 120 and 80 cm spacing, and four central rows were used for evaluations at 40 cm spacing.

Sowing was carried out on 16th March, 18th March and 2nd April, 2004, in Pedrinhas Paulista, Cândido Mota, and Palmital, respectively. The seeds were treated with thiodicarb + imidacloprid insecticides. Fertilization was carried out using 16, 40, and 40 kg·ha^{-1} of N, P_2O_5, and K_2O, respectively. Two seeds were planted per hole, and the plants were thinned six days after emergence (DAE) to obtain definitive populations. Nitrogen (40 kg·ha^{-1}) in the form of ammonium sulfate was used in topdressing fertilization, and insecticides were sprayed to control the fall armyworm (*Spodoptera frugiperda*).

Specifically, in Palmital, there was frost on the 14th day of June (73 DAE), immediately before flowering of the plants, with temperature of 3 °C (Fig. 1). At 88 DAE, at the beginning of grain filling (stage R3), ten plants were sampled in the useful area of each plot. The length and width of all leaves were measured, and the leaf area (LA) of each plant and leaf area index (LAI) of each plot were calculated. The percentage of leaf area burned by frost was visually evaluated by estimative, as a percentage of the total leaf area, by a single evaluator.

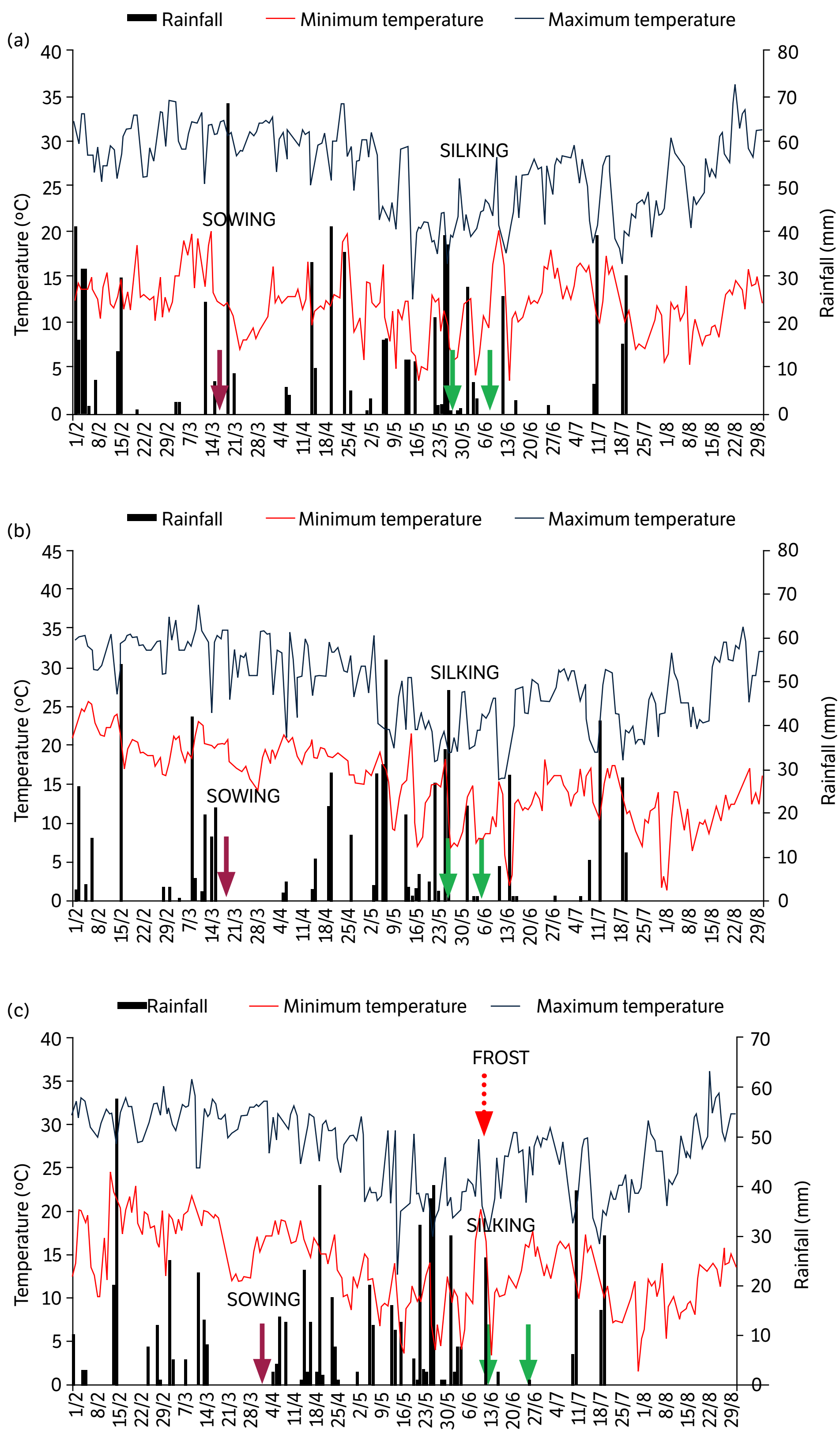

Figure 1. Rainfall and minimum and maximum daily temperatures, from February to August 2004, and arrows indicating the dates of sowing and silking of the plants, in (a) Cândido Mota, (b) Pedrinhas Paulista and (c) Palmital, São Paulo, Brazil.

The leaf area of each leaf was obtained from the Eq. 1, proposed by Tollenaar (1992):

$$LA = LL \times LW \times 0.75 \quad (1)$$

where: LL: leaf length (cm); LW: leaf width (cm).

LAI was calculated based on the relationship between the leaf area of the 10 plants and the area occupied by the plants on the soil based on the density and population arrangement of the plot. The effect of frost was evaluated by estimating the percentage of leaf area burned on each leaf. For that, we multiplied the leaf area of each leaf by the necrotic proportion and calculated the proportion of the affected area in all the leaves in relation to the plant leaf area. Finally, it was calculated the average percentage of the leaf area affected in the 10 plants evaluated in the plot.

The soluble solid contents in the stalk (brix), height until the insertion of the last leaf, and height of the first ear were evaluated in the same 10 plants, and the average values per plot were recorded. Brix was determined at the 5th internode, counting from the base towards the apex of the plant, using a direct reading manual refractometer. The stalk diameter was measured with a caliper, at the widest part of the third internode. The dry matter of plant shoots and the fractionation of the total dry matter in leaves, stalk, and ears were also evaluated. All the plant fractions were weighed and ground, and a sample of 500 g was taken to determine the dry matter content (oven at 60 °C for 72 h).

In the three trials, the ears were harvested when the grain moisture was equal to or less than 21%. We evaluated the height of plants, total number of plants and ears, number of stalk lodging, mass of ears with husk and, after threshing, mass and moisture of the grains. Thereafter, we calculated the number of ears per plant, percentage of stalk lodging, percentage of grains in the ear, and the yield corrected for 13% moisture. In Palmital, we used the average heights of plants and ears obtained immediately after the occurrence of frost.

The experimental data were analyzed by analysis of variance (ANOVA) with the SAS program (Littel et al. 1996). Due to the great variability among environments, only individual analyses per location were performed. Population and seeding density were compared using polynomial regression and cultivar comparison were made using Tukey's test, both at the 5% level of significance.

Rainfall and minimum and maximum daily temperatures data were collected from the automated agrometeorological stations of the Agronomic Institute, located up to 5 km from the experiments. Ten-day water balances were constructed according to the method proposed by Thornthwaite and Mather (1955) and modified by Camargo and Camargo (1983)[1]. The available water capacity in the soil was considered equal to 50 m.

RESULTS AND DISCUSSION

Agronomic and morphological parameters

Higher average yields were observed in the experiments conducted at Cândido Mota and Pedrinhas Paulista (4,604, 4,570 $kg \cdot ha^{-1}$, respectively), where corns were sown in mid-March, than in Palmital (2,331 $kg \cdot ha^{-1}$), which was sowed in early April and frost occurred (Table 1). Late sowing is the main cause of low yields in the Médio Paranapanema region, according to Duarte et al. (2017). Water deficit occurred in almost the entire month of March to mid-April, during the initial stages of corn development, that conditioned small plants (height of plants less than 2 m), and several days of minimum temperatures below 10 °C from the second 10 days of May to the first 10 days of June (Fig. 1). The few rains that occurred in March provided sufficient soil moisture for plant emergence. Notably, flowering occurred in mid-June in Palmital and early June in the other two locations, before the long period of water deficit from the third 10 days of June (water balance data not shown).

[1] Camargo, A. P. and Camargo, M. B. P. (1983). Teste de uma equação simples para estimativa da evapotranspiração potencial baseada na radiação solar extraterrestre e na temperatura do ar. In: Congresso Brasileiro de Agrometeorologia, 3. Campinas. Anais... Campinas: Sociedade Brasileira de Agrometeorologia. p. 229-244.

Table 1. Means of agronomic parameters and results of variance analysis of the factors cultivars, row spacings and plant populations, in Cândido Mota, Pedrinhas Paulista and Palmital, São Paulo, Brazil.

Cultivar	Height of plants			Stalk lodging[1]			Ears per plant			Grains in the ear			Yield		
	C. Mota	Pedrinhas	Palmital	C. Mota	Pedrinhas	Palmital	C. Mota	Pedrinhas	Palmital	C. Mota	Pedrinhas	Palmital	C. Mota	Pedrinhas	Palmital
	.... cm			.... %			.... cm			.... %			.... $kg \cdot ha^{-1}$		
Cultivar															
Fort	182 a	190 a	186 a	29 a	36 a	87 a	0,98	1.01	1.01	74 a	76 a	68 a	5,046 a	4,763 a	2,554 a
DKB 950	156 b	173 b	158 b	1 b	3 b	21 b	0,99	1.01	1.00	70 b	73 b	67 b	4,162 b	4,377 b	2,108 b
Row spacing															
40	169	181	170	16	28	57	1,01	1.01	1.01	72	74	68	4,92	4,431	2,354
80	169	182	173	14	17	53	0,97	1.01	1.00	72	74	67	4,51	4,567	2,356
120	168	183	173	15	15	53	0,98	1.01	0.99	72	75	68	4,382	4,712	2,283
Plant population															
25,000	160	180	165	2	1	33	1,07	1.05	1.02	71	74	67	3,366	3,814	1,873
43,750	171	180	169	8	11	48	1,00	0.98	1.00	73	75	68	4,812	4,657	2,376
62,500	172	183	175	22	27	61	0,95	1.00	1.00	72	75	68	4,876	4,996	2,486
81,250	173	184	177	28	40	76	0,93	1.02	0.99	72	74	68	5,362	4,813	2,589
Cultivar (C)	**	**	**	**	**	**	ns	ns	ns	**	**	**	**	**	**
Row spacing (S)	ns	ns	ns	ns	**	ns	ns	ns	ns	ns	ns	ns	**	**	ns
linear	ns	ns	*	ns	**	ns	ns	ns	*	ns	ns	ns	**	**	ns
quadratic	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Plant population (P)	**	ns	**	**	**	**	**	*	ns	**	ns	**	**	**	**
linear	**	*	**	**	**	**	**	ns	*	**	ns	**	**	**	**
quadratic	**	ns	ns	ns	ns	ns	ns	**	ns	**	ns	**	**	**	**
C x S	ns	**	*	ns	ns	ns	ns	ns	ns	ns	ns	**	ns	**	*
C x P	**	ns	ns	**	ns	**	*	ns	ns	ns	ns	**	ns	ns	ns
S x P	ns	ns	ns	*	*	*	ns	ns	ns	ns	**	ns	ns	ns	ns
C x S x P	ns	ns	*	*	ns	ns	ns	ns	ns	ns	ns	**	ns	ns	**
Block	**	ns	ns	ns	ns	ns	ns	ns	ns	ns	*	**	ns	ns	**
C.V.%	3.7	7.0	3.7	37.4	38.7	11.3	10.5	7.2	3.4	1.6	2.1	1.7	10.6	6.0	10.2

[1] Analyzed by (x + 0,5)1/2; *p ≤ 0.05; **p ≤ 0.01; ns: not significant; lowercase letters into each column indicate differences between cultivar according to Tukey's test (p ≤0.05); C.V.: coefficient of variation.

There was a cultivar effect in the three locations for most parameters, except for the ears per plant (Table 1). Fort had the highest plant size, stalk lodging index, grains in the ear, and grain yield. As for the parameters specifically evaluated in Palmital, the hybrid Fort showed higher stalk diameter, LAI, and proportion of stalk and leaves in the mass than DKB 950, while the DKB 950 had higher brix in the stem and proportion of ears in the mass (Table 2). The higher value of ear fraction in DKB 950 confirmed its greater precocity compared to that of Fort, as the evaluation was carried out a few days after flowering.

Spacing reduction provided antagonistic effects in Cândido Mota and Pedrinhas Paulista, increasing and decreasing yield linearly, respectively (Table 1). However, in Pedrinhas Paulista, the spacing effect occurred only in the Fort, with greater plant height and yield in the 120 cm spacing (Fig. 2). In Palmital, where the yield was very low due to the occurrence of frost, analysis of the interaction between cultivar and row spacing showed that the hybrid DKB 950 had a greater yield in spacing close to 80 cm (quadratic effect) and the yield of Fort did not differ among spacings (Fig. 3). The literature indicates greater responses to rows spacing reduction in cultivars with more erect leaf architecture and shorter plant cycle in specific off-season corn environments (Penariol et al. 2003, Kappes et al. 2011). However, this relationship was not found in the summer season (Sangoi et al. 2001, Greveniotis et al. 2019) and off-season (Dias et al. 2019).

Table 2. Characterization of maize plants in the R3 stage and the effect of frost on the reduction of the green leaf area index, immediately after its occurrence, as a function of cultivars, row spacings and plant populations, in Palmital, São Paulo, Brazil.

Cultivar	Stalk		Leaf area index		Dry matter	Plant fractionation		
	diameter	brix	total	burned (1)		leaves	stalk	ears
	.... mm		.... %		kg·ha^{-1}	.... %		
Cultivar								
Fort	200 a	8.9 b	3.0 a	21 a	5,615	29 a	51 a	20 b
DKB 950	189 b	9.9 a	2.4 b	16 b	5,343	26 b	47 b	27 a
Row spacing								
40	197	8.7	2.8	21	5,438	28	51	21
80	194	10.1	2.6	19	5,919	26	47	27
120	193	9.4	2.6	14	5,081	27	50	23
Plant population								
25,000	234	9.4	1.5	17	4,015	25	46	29
43,750	203	9.2	2.3	19	5,182	27	49	24
62,500	179	9.4	3.1	18	6,061	28	50	22
81,250	162	9.5	3.8	19	6,659	28	53	19
Cultivar (C)	**	**	**	**	ns	**	**	**
Row spacing (S)	ns	**	**	**	**	**	**	**
linear	**	**	**	**	ns	**	ns	**
quadratic	**	**	*	ns	**	*	**	**
Plant population (P)	**	ns	**	ns	**	**	**	**
linear	**	ns	**	ns	**	**	**	**
quadratic	**	ns	ns	ns	ns	**	ns	ns
C x S	ns	*	ns	ns	*	**	ns	**
C x P	ns	**	**	ns	ns	ns	ns	ns
S x P	ns	**	ns	ns	*	**	**	**
C x S x P	ns	**	ns	ns	ns	ns	ns	ns
Block	ns	ns	**	**	ns	ns	ns	ns
C.V.%	5.8	6.6	9.3	18.1	16.2	7.2	6.3	12.8

[1]Analyzed by $x^{1/2}$; *$p < 0.05$; **$p < 0.01$; ns: not significant; lowercase letters into each column indicate differences between cultivar according to Tukey's test ($p \leq 0.05$); C.V.: coefficient of variation.

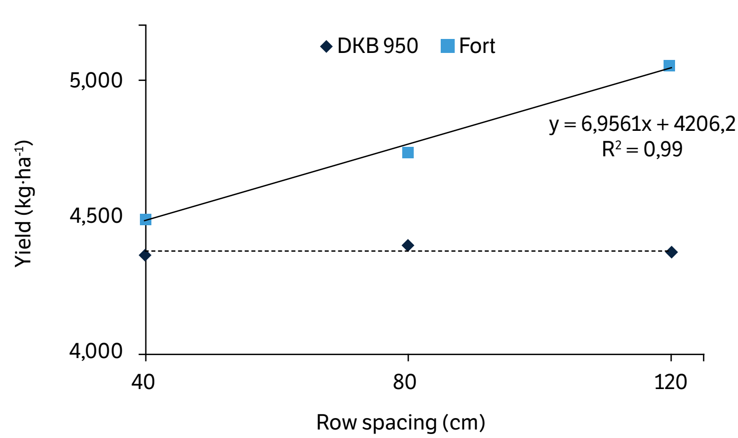

Figure 2. Effect of row spacings on grain yield, in the cultivars DKB 950 and Fort, in Pedrinhas Paulista, São Paulo, Brazil.

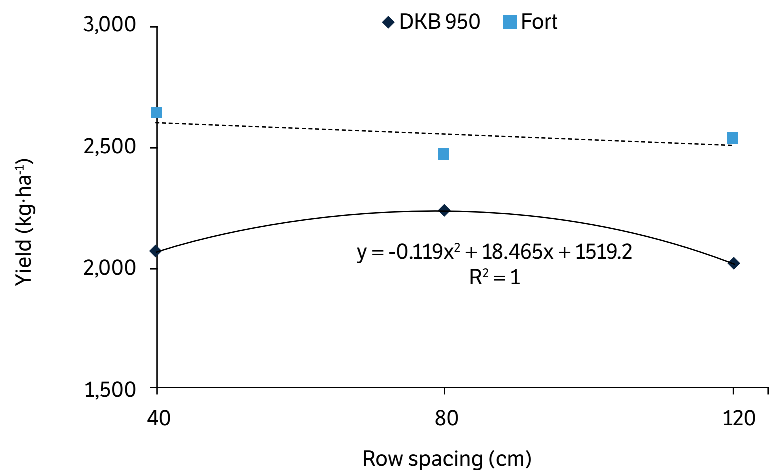

Figure 3. Effect of row spacings on grain yield, in the cultivars DKB 950 and Fort, in Palmital, São Paulo, Brazil.

In Palmital, there was a quadratic effect of spacing on brix, shoot dry mass, and proportions of ears, stalk, and leaves in plant mass (Table 2). The effects were different in each hybrid.

Lower plant mass at 40 cm compared to 80 cm spacing and a clear interaction between spacing versus cultivar were observed. DKB 950 and Fort showed the highest and lowest values of plant mass in narrow spacings, respectively (Fig. 4). The values of Brix and proportion of mass of ears were higher in the intermediate rows spacing and more with a greater reduction of the spacing from 80 to 40 cm in the Fort hybrid, and the cultivar Fort was the only one that increased the proportion of leaves of plants with reduced spacing to the detriment of the ear fraction (Figs. 5, 6 and 7). Thus, the plants had less reserves in the stems in the form of sugars in the smaller spacing since there was no difference in plant height and stem diameter between the spacing, which could change the amount stored.

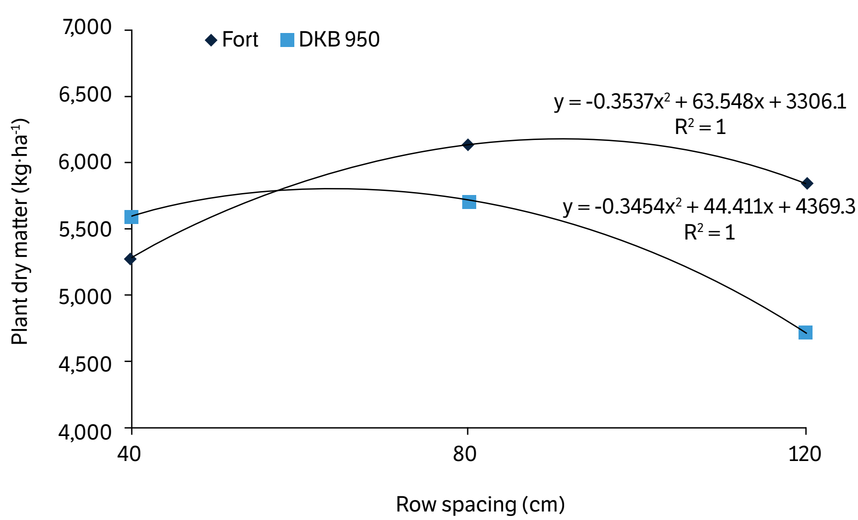

Figure 4. Dry matter of plant shoots, in the stage R3, in function of the row spacings, in the cultivars DKB 950 and Fort, in Palmital, São Paulo, Brazil.

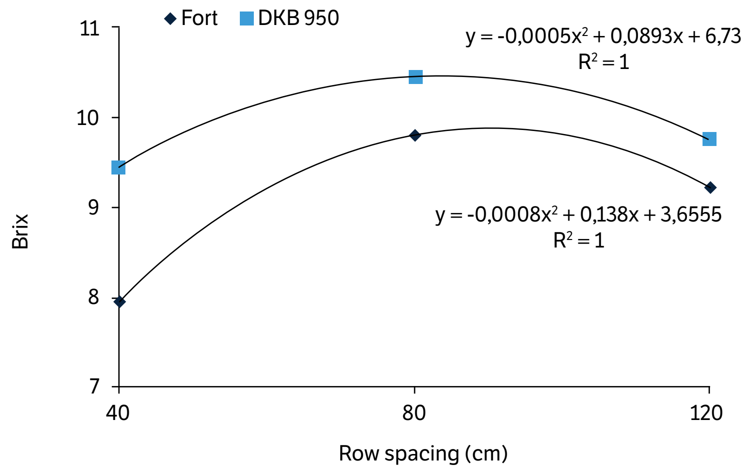

Figure 5. Brix values, at stage R3, as a function of row spacings, in the cultivars DKB 950 and Fort, in Palmital, São Paulo, Brazil.

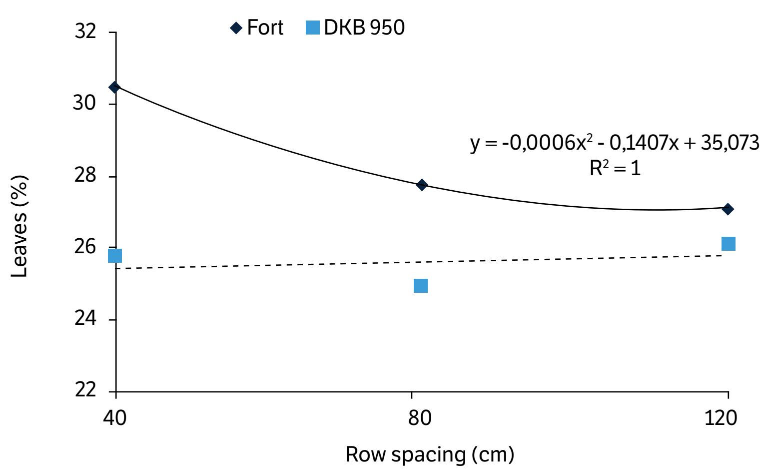

Figure 6. Percentage of leaves in the dry mass of plant shoots, in the R3 stage, as a function of the row spacings, in the cultivars DKB 950 and Fort, in Palmital, São Paulo, Brazil.

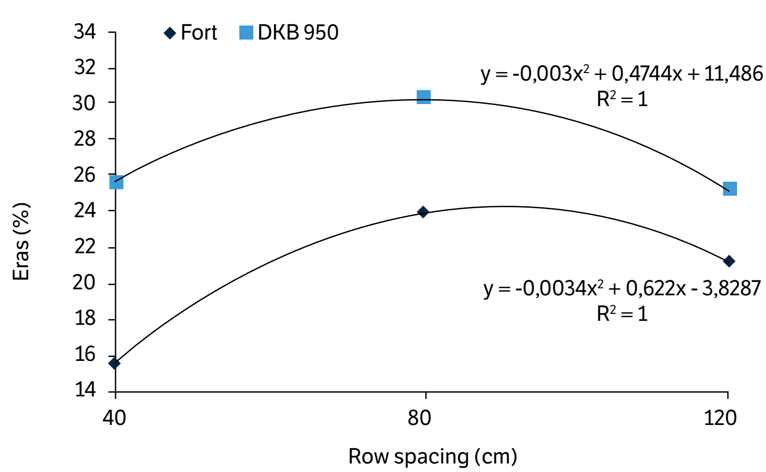

Figure 7. Percentage of ears in the dry mass of plant shoots, in the R3 stage, as a function of the row spacings, in the cultivars DKB 950 and Fort, in Palmital, São Paulo, Brazil.

LAI values were very low (1.5 to 3.8) compared to that reported by Dias et al. (2019), under off-season conditions; those obtained by Sangoi et al. (2001) and Graffitti et al. (2021), in the second crop irrigated and summer crop in Brazil, respectively; and reported by Ahmad et al. (2010), Bernhard and Below (2020) and Djaman et al. (2022) in other countries, with values usually close to or greater than 5. The lack of rain and the consequent water deficit before flowering (Fig. 1) contributed to the stunting of the plants (1.86 and 1.58 in the cultivars Fort and DKB 950, respectively) (Table 1) and probably to the low leaf development. Alves et al. (2013) obtained plants with low size (average = 2 m) and LAI (average at the R4 stage = 3.1) associated with low yields (average = 7.3 Mg·ha^{-1}) under off-season conditions.

LAI was greater at 40 cm spacing and markedly reduced at 80 cm spacing, staying practically constant between 80 and 120 cm (quadratic effect) (Fig. 4). However, the highest LAI values at 40 cm spacing did not provide an increase in dry mass and grain yield compared to that at 80 cm (Figs. 3 e 5). It is important to mention that Dias et al. (2019) and Graffitti et al. (2021) did not reported change in LAI after silking with planting row reduction. According to Strieder et al. (2008), LAI may vary with row spacing, but their effects depend on density, type of plant, management system and phenological stage.

In Palmital, an interaction was observed between the population and rows spacing for the dry mass of plants (Fig. 8). The spacing effect was not significant in the lowest population (25,000 plants·ha^{-1}), and there was no reduction in dry mass at 40 cm spacing compared to 80 cm in the highest population (81,250 plants·ha^{-1}), in contrast to populations of 43,700 and 62,500 plants·ha^{-1}. Therefore, the negative effect of rows spacing reduction on mass production was observed only in the cultivar Fort and the intermediate populations. Moreover, the yield results did not always follow those of the mass, because, as already mentioned, the yield of the cultivar Fort did not vary with spacing at Palmital.

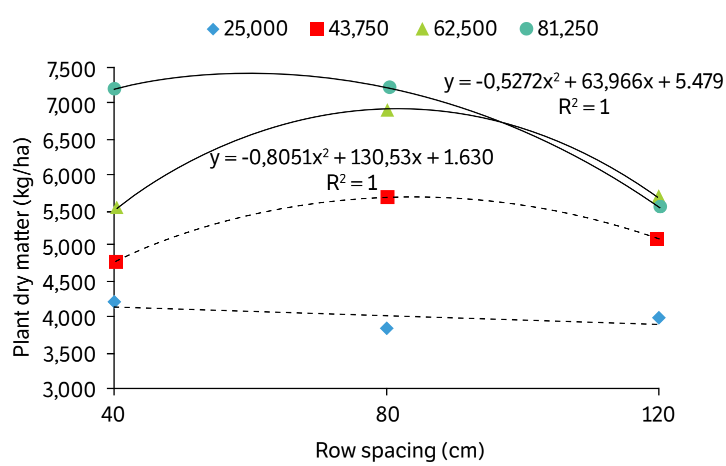

Figure 8. Dry mass of plant shoots, in the R3 stage, as a function of the row spacings and plant populations (25,000, 43,750, 62,500 and 81,250 plants per hectare), in Palmital, São Paulo, Brazil.

The reduction in spacing increased the rate of stalk lodging in Pedrinhas Paulista, corroborating the results of Schwantes et al. (2007). However, the occurrence of stalk lodging plants increased only in the largest populations (62,500 and 81,250 plants·ha^{-1}) (data not shown). This was the only place with water deficit in the third 10-day period of April, extending the initial period of water stress until the V6 stage. Notably, observed the yield reduction in narrow rows even all ears of the useful plot have been harvested, including broken plants. Probably, in this condition of accentuated hydric stress, rotting stalks that is associated to stalk lodging interfered in grain filling. Considering that there is a trend towards an increase in the population of plants in off-season corn crops (Duarte 2022), the negative effect of spacing reduction on the increase in stalk lodging may be relevant when populations are equal or greater to 63,000 plants·ha^{-1}.

The population factor changed almost all agronomic variables, except for plant height and grains in the ear in Pedrinhas Paulista and ear per plant in Palmital (Tabela 1). Population density increased plant size and plant breakage and reduced the number of ears per plant, corroborating the reports of Penariol et al. (2003) and Carmo et al. (2020). However, the hybrid DKB 950 did not increase stalk lodging in Cândido Mota and had this problem only under high plant populations in Palmital, contrary to the hybrid Fort (data not shown).

The optimum plant density that maximizes grain yield varied with environment, in accordance with Djaman et al. (2022), but there was no interaction between cultivars and plant density. Yield increased quadratically with increasing plant population, with maximum values of 75,000, 68,071, and 70,717 plants per hectare in Cândido Mota, Pedrinhas Paulista, and Palmital, respectively. These values are high compared to the 55 to 65 thousand plants per hectare used in crops in this yield range (Duarte et al. 2017, Duarte 2022). One of the risks of using high plant populations is the occurrence of broken plants (Duvick 2005, Ma et al. 2014), as occurred in the present study.

In the evaluations carried out in Palmital, the dry mass, LAI, and the proportion of plant leaves and stems increased as a result of the increase in plant population, but the stem diameter and proportion of ears in the plant decreased (Table 2), corroborating the results of Dias et al. (2019) and Graffitti et al. (2021). Several density and population arrangement studies showed that an increase in leaf area might not ensure greater yield (Bernhard and Below 2020, Dias et al. 2019, Djaman et al. 2022, Graffitti et al. 2021, Overman and Scholtz 2011), especially in genotypes with open architecture (Ahmad et al. 2010).

Brix was the only parameter that was not influenced by plant population, even with the unfolding of the interaction between population and cultivar (data not shown). The reduced stem diameter resulting from the increase in the population density may have decreased the storage of sugars in the stem of each plant, as was inferred for the reduction of spacing. Therefore, the increase in plant mass per area and LAI as a result of the increase in plant population may have occurred concomitantly with the reduction in the accumulation of reserves in each plant, consequently limiting ear development.

Effect of frost on leaf area and yield

The frosts caused an average damage of 18% in the leaf area, with greater damage in the upper third of the plants, mainly in the apices of the leaves. In corn, the leaves are the organs where the injuries from cooling are first observed, as they undergo cellular autolysis and tissue necrosis for being less lignified. However, non-necrotic tissues are also negatively impacted by cooling (Bilska and Sowinski 2010). As temperatures re-established after the frost, the maintenance of part of the green leaves allowed the plants to continue developing until grain maturity (Carter, 1995). However, the yield potential was compromised by the water deficit in the vegetative phase, frost, and low availability of heat and direct solar radiation of the winter equinox in this region (Duarte and Kappes 2015).

The hybrid Fort showed greater frost damage probably due to the higher LAI and leaf angle compared to the stem. However, under the conditions in which the field trial was carried out, it was the most adapted hybrid in Palmital in terms of yield potential.

In reduced spacings, the effects of frost were greater without interaction between spacing versus hybrids and spacing versus plant density (Table 2). This may be because higher LAI provided greater soil surface coverage between the sowing rows, reducing the fluxes in and out of soil heat. A greater vegetation cover would have resulted in a lower flux of radiation emitted by the soil during the night and, consequently, a more intense cooling of the plants (Loss 1987).

As already mentioned by Palmital, grain yield was lower at 120 cm spacing in the hybrid DKB 950, following the same trend of dry mass, but with a slight decrease at 40 cm spacing compared to at 80 cm spacing (Fig. 3). This may be because of the greater frost burn at 40 cm spacing (21% compared to 14% at 80 cm), canceling out the possible advantage of the greater LAI under reduced spacing (2.83 compared to 2.62 at 80 cm).

The greater burning of the leaves by frost in the 40 cm spacing, in relation to the 80 cm, reinforces the inference that the reduction of the spacing to 40 cm does not guarantee better yield in conditions of low productivity caused by environmental stress. Furthermore, the absence of effect of plant population on leaves frost burn allows to confirm that the most limiting factor for population density is the increase in plant breakage. Thus, cultivars such as the hybrid DKB 950, smaller in size and with more erect leaves, which had less foliar damage from frost and lower indices of broken plants, could benefit from reduced spacing up to 40 cm and higher population density in some environments.

CONCLUSION

The spacing reduction provided antagonistic effects on agronomic parameters by local, with different response from hybrids, not ensuring better yields.

The greater damage by moderate frosts in reduced spacing must be taken into account when choosing the spacing of off-season corn production in areas with frequent frost.

Populations equal to or greater than 68,000 plants per hectare provide the best yields, regardless of the occurrence of frost, but they can result in high rates of stalk lodging in some cultivar.

AUTHORS' CONTRIBUTION

Conceptualization: Duarte, A. P.; **Methodology:** Duarte, A. P.; **Investigation:** Duarte, A. P. and Duarte, R. C. R. M.; **Writing – Original Draft:** Duarte, A. P.; **Writing – Review and Editing:** Duarte, A. P. and Duarte, R. C. R. M.; **Supervision:** Duarte, A. P.

DATA AVAILABILITY STATEMENT

All dataset were generated and analyzed in the current study.

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REFERENCES

Ahmad, M., Khaliq, A., Ahmad, R. and Ranjha, A. M. (2010). Allometery and productivity of autumn planted maize hybrids under narrow row spacing. International Journal of Agriculture & Biology, 12, 661-667.

Alves, V. B., Ceccon, G. and Leite, L. F. (2013). Morfologia e produtividade de híbridos de milho safrinha solteiro e consorciado com braquiária. Revista Brasileira de Milho e Sorgo, 12, 152-163. https://doi.org/10.18512/1980-6477/rbms.v12n2p152-163

Bernhard, B. J. and Below, F. E. (2020). Plant population and row spacing effects on corn: Plant growth, phenology, and grain yield. Agronomy Journal, 112, 2456-2465. https://doi.org/10.1002/agj2.20245

Bilska, A. and Sowinski, P. (2010). Closure of plasmodesmata in maize (Zea mays) at low temperature: a new mechanism for inhibition of photosynthesis. Annals of Botany, 106, 675-686. https://doi.org/10.1093/aob/mcq169

Carmo, E. L., Rocha, A. G. C., Ferreira, C. J. B., Ribeiro Junior, L. F., Simon, G. A., Sousa, J. V. A. and Vieira Neto, D. F. (2020). Arranjos espaciais de plantas de milho cultivado em condições de Cerrado, Semina: Ciências Agrárias, 41, 1483-1494. https://doi.org/10.5433/1679-0359.2020v41n5p1483

Choudhury, B. U., Webster, R., Sharma, V., Goswami, J., Meetei, T. T., Krishnappa, R. and Raju, P. L. N. (2019). Frost damage to maize in northeast India: assessment and estimated loss of yield by hyperspectral proximal remote sensing. Journal of Applied Remote Sensing, 13, 044527. https://doi.org/10.1117/1.JRS.13.044527

[CONAB] Companhia Nacional de Abastecimento (2022). Acompanhamento de safra brasileiro – grãos: safra 2021/22. Brasília: Companhia Nacional de Abastecimento. Available at: https://www.conab.gov.br/info-agro/safras/graos/boletim-da-safra-de-graos. Accessed on: May 3, 2022.

Carter, P. R. (1995). Late-spring frost and postfrost clipping effects on corn growth and yield. Journal of Production Agriculture, 8, 203-209. https://doi.org/10.2134/jpa1995.0203

Cruz, J. C., Pereira, F. T. F., Pereira Filho, I. A., Oliveira, A. C. and Magalhães, P. C. (2007). Resposta de cultivares de milho à variação em espaçamento e densidade. Revista Brasileira de Milho e Sorgo, 6, 60-73. https://doi.org/10.18512/1980-6477/rbms.v6n01p%25p

Dias, F. S., Rezende, W. M., Zuffo, L. T., Caixeta, D. G., Massensini, M. A., Ribeiro Junior, J. I. and Delima, R. O. (2019). Agronomic responses of maize hybrids to row spacing and plant population in the summer and winter seasons in Brazil. Agronomy Journal, 111, 3119-3129. https://doi.org/10.2134/agronj2018.12.0765

Djaman, K., Allen, S., Djaman, D. S., Koudahe, K., Irmak, S., Puppala, N., Darapuneni, M. K. and Angadi, S. V. (2022). Planting date and plant density effects on maize growth, yield and water use efficiency. Environmental Challenges, 6, 100417. https://doi.org/10.1016/j.envc.2021.100417

Duarte, A. P. (2022). Adubação do milho-safrinha em sucessão à soja. In D. C. Abreu, W. M. Silva, D. T. Pinheiro and A. R. R. Martins (Eds.), 3ª Vitrine Tecnológica Agrícola: atualidades na cultura do milho em sistema soja e milho-safrinha (p. 15-33). Cuiabá: Uniselva.

Duarte, A. P. and Kappes, C. (2015). Evolução dos sistemas de cultivo de milho no Brasil. Informações Agronômicas, (152), 15-18.

Duarte, A. P., Fantin, G. M., Michelotto, M. D., Hirata, A. C., Sawazaki, E., Cantarella, H. and Paterniani, M. E. A. G. (2017). Sistemas de produção de milho safrinha e programa de melhoramento de milho do IAC. In A. P. Duarte and R. A. D. Kanthack (Eds.), Tecnologias desenvolvidas pela APTA na região do Médio Paranapanema (p. 121-168). Campinas: Instituto Agronômico.

Duvick, D. N. (2005). Genetic progress in yield of United States maize (Zea mays L.). Maydica, 50, 193-202.

Graffitti, M. S., Umburanas, R. C., Fontana, D. C., Pilau, F. G., Reichardt, K. and Dourado Neto, D. (2021). Performance of maize hybrids as a function of spatial arrangements during second growth season under irrigation. Bragantia, 80, e2321. https://doi.org/10.1590/1678-4499.20200498

Greveniotis, V., Zotis, S., Sioki, E. and Ipsilandis, C. (2019). Field Population Density Effects on Field Yield and Morphological Characteristics of Maize. Agriculture, 9, 160. https://doi.org/10.3390/agriculture9070160

Harwood, J. (1991). Strategies for coping with low environmental temperatures. Trends in Biochesmistry Sciences, 16, 126-127. https://doi.org/10.1016/0968-0004(91)90052-W

Kappes, C., Andrade, J. A. C., Arf, O., Oliveira, A. C., Arf, M. V. and Ferreira, J. P. (2011). Desempenho de híbridos de milho em diferentes arranjos espaciais de plantas. Bragantia, 70, 334-343. https://doi.org/10.1590/S0006-87052011000200012

Littel, R. C., Milliken, G. A., Stroup, W. W. and Wolfinger, R. D. (1996). SAS. System for mixed models. Cary: SAS Institute.

Loss, S. P. (1987). Factors affecting frost damage to wheat in western Australia. Division of Plant Research, Technical Report, nº 6.

Ma, D., Xie, R., Liu, X., Niu, X., Hou, P., Wang, K., Lu, Y. and Li, S. (2014). Lodging-Related Stalk Characteristics of Maize Varieties in China since the 1950s. Crop Science, 54, 2805-2814. https://doi.org/10.2135/cropsci2014.04.0301

Overman, A. R. and Scholtz, R. V. (2011). Model of yield response of corn to plant population and absorption of solar energy. PLoS One, 6, e16117. https://doi.org/10.1371/journal.pone.0016117

Pantano, A. P. (2017). Levantamento climático em Assis no período 1992 a 2016 In A. P. Duarte and R. A. D. (Eds.), Tecnologias desenvolvidas pela APTA na região do Médio Paranapanema (p. 51-58). Campinas: Instituto Agronômico.

Penariol, F. G., Fornasieri Filho, D., Coicev, L., Bordin, L. and Farinelli, R. (2003). Comportamento de cultivares de milho semeadas em diferentes espaçamentos entre linhas e densidades populacionais, na safrinha. Revista Brasileira de Milho e Sorgo, 2, 52-60. https://doi.org/10.18512/1980-6477/rbms.v2n02p%25p

Sangoi, L., Ender, M., Guidolin, A. F., Almeida, M. L. and Heberle, P. C. (2001). Influence of row spacing reduction on maize grain yield in regions with a short summer. Pesquisa Agropecuária Brasileira, 36, 861-869. https://doi.org/10.1590/S0100-204X2001000600003

Schwantes, D. O., Schuelter, A. R., Fontoura, D. and Marochio, J. (2007). Características agronômicas de híbridos de milho sob espaçamento reduzido entre linhas: Safrinha de 2005. Scientia Agraria, 8, 343-349. https://doi.org/10.5380/rsa.v8i3.8594

Strieder, M. L., Silva, P. R. F., Argenta, G., Rambo, L., Sangoi, L., Silva, A. A. and Endrino, P. C. (2007). A resposta do milho irrigado ao espaçamento entrelinhas depende do híbrido e da densidade de plantas. Ciência Rural, 37, 634-642. https://doi.org/10.1590/S0103-84782007000300006

Strieder, M. L., Silva, P. R. F., Rambo, L., Bergamaschi, H., Dalmago, G. A., Endrigo, P. C. and Jandrey, D. B. (2008). Características de dossel e rendimento de milho em diferentes espaçamentos e sistemas de manejo. Pesquisa Agropecuária Brasileira, 43, 309-317. https://doi.org/10.1590/S0100-204X2008000300004

Thornthwaite, C. W. and Mather, J. R. (1955). The water balance. New Jersey: Drexel Institute of Technology, Laboratory of Climatology.

Tollenaar, M. (1992). Is low density a stress in maize? Maydica, 37, 305-311.

Zaidi, P. H., Yadav, M., Maniselvan, P., Khan, R., Shadakshari, T. V., Singh, R. P. and Pal, D. (2010). Morpho-physiological traits associated with cold stress tolerance in tropical maize (*Zea Mays* L.). Maydica, 55, 201-208.

Zhai, L., Xie, R., Li, S. and Fan, P. (2017). Relationship between population competitive intensity and yield in maize cultivars. Journal of Integrative Agriculture, 16, 1312-1321. https://doi.org/10.1016/S2095-3119(16)61541-1

Zhou, X., Muhammad, I., Lan, H. and Xia, C. (2022). Recent Advances in the Analysis of Cold Tolerance in Maize. Frontiers in Plant Science, 13, 866034. https://doi.org/10.3389/fpls.2022.866034