Influence of row spacing on canopy and seed production in grain amaranth (*Amaranthus cruentus* L.)

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Abstract. A new crop recently introduced in Italy is amaranth. Studies involving agronomic techniques on this plant are limited. The aim of the present research was to assess the effect of distance between rows on both seed yield and ground cover in *Amaranthus cruentus* L. Sowing treatments included two single row spacing designs (18 and 60 cm) and one double row spacing design (18 + 60 cm). At the six true leaf stage, in the single row design of 60 and 18 cm row spacing, ground cover was 16% and 47% respectively. An intermediate coverage of 31% was evident in the double rows. At the ten true leaf stage, plants cultivated in single rows at 18 cm covered the ground early, thereby attaining a ground cover of 85%. Regarding yield, a seed production of 0.92 t ha^{-1} was obtained from plants in the double row design compared to the respective single row spacing designs of 18 and 60 cm, where yields were 0.85 and 0.70 t ha^{-1} respectively. The selection of one mode of sowing over another will largely depend on the type of equipment available to the farm. Whilst single row spacing distances of 18 cm displayed a net of advantage against weeds, difficulties were encountered in the case of managing weeds by mechanical equipment. The use of double rows permitted taking advantage of a slightly better ground cover than single rows, together with the possibility of mechanical intervention for the control of weeds, and importantly also provided a higher yield.

Key words: grain amaranth, *Amaranthus cruentus*, row spacing, Central Italy.

INTRODUCTION

The globalization and industrialization of agriculture on a worldwide scale has led to a number of deep changes in the sector, many of which have negative effects. These include the development of technologies geared towards crops with a high demand for fertilizers, water and pesticides (herbicides, fungicides and insecticides) and, therefore, a high-energy demand. Moreover, the widespread diffusion of monocultures over time has resulted in a significant reduction in biodiversity, also within the agricultural sector, as well as problems of soil fertility and weed- and pathogen-induced resistance.

Within the framework of challenges facing Italian agriculture, particularly that of the hilly areas of Central Italy, traditional industrial food crops are increasingly less attractive from an economic point of view (Casini & La Rocca, 2012). Farmers are faced with difficulties in selecting crops that secure an income. Moreover, given that the average size of the farms is limited, the choices and interests of farmers are oriented towards to 'new crops' to secure a market outlet. These market outlets are not only

reflected in products for celiac patients, but also in pharmaceutical, herbal and nutraceutical products in general. Currently, research interest in these crops, relating to both resurgence and enhancement, is now a strongly acquired trend in both scientific and economic sectors.

New seed crops, recently introduced in Italy, include quinoa (*Chenopodium quinoa* Willd.) and amaranth (*Amaranthus cruentus* L. and *Amaranthus hypochondriacus* L.). Although a small supply chain production has already been initiated for quinoa, the situation for grain amaranth is still at the starting point (Lovelli et al., 2005; Rivelli et al., 2008; Casini & La Rocca, 2015). The most common studies conducted thus far are related to the adaptability of different species of amaranth, as well as genotype characterization (Ercoli et al., 1987; Massantini et al., 1987; Alba et al., 1997; Casini et al., 2012; El Gendy et al., 2018). Studies involving agronomic techniques are limited (Ercoli et al., 1987; Casini & La Rocca, 2014; Pulvento et al., 2015).

Research conducted on different row space distances and in different climatic conditions has produced contrasting results. In some cases, variable distances ranging from 30 and 76 cm did not produce any significant effect on seed yield (Robinson 1986; Putnam 1990; Kauffman, 1992; Tracey et al., 2000; Nurse et al., 2016). In other studies, production increases and decreases were attained depending on row space differences, often also in relation to density or the cultivation environment (Misra et al., 1985; Svirskis, 2003; Rotich et al., 2017). Yield increases were reported with narrow row spacing ranging between 30 and 36 cm (Endres 1986; Jamriška 1998; Chaudhari et al., 2009; Olofintoye et al., 2011), whereas a decrease was reported by Misra et al. (1985).

No specific studies have yet been carried out in Italy. Initial research included the use of variable row space distances ranging from 50 to 70 cm. For the distance set at 50 cm, different plant densities were investigated and preliminary results indicated that the best yields were obtained with 30 and 60 plants m^{-2} (Casini & La Rocca, 2014).

Unlike in conventional agriculture, the use of double row spacing is very common in organic agriculture. In the latter, particularly for winter cereals, double row spacing is technique that facilitates the mechanical control of weeds, and it derives from an ancient technique, widespread in Italy when weeds were removed manually.

The aim of the present research was to assess the effect of row spacing (single or double) using the best plant density as observed by previous trial (Casini & La Rocca, 2014) either seed yield or ground cover in *Amaranthus cruentus* L. in Central Italy.

MATERIALS AND METHODS

A field experiment was carried out in 2019 in Tuscany, Central Italy at the 'Centro per il Collaudo ed il Trasferimento dell'Innovazione di Cesa (Arezzo)' (43°18' north; 11°47' east, 246 m asl), on a neutral, loamy-sandy soil. The physical and chemical characteristics of the soil (depth of 20 cm) were as follows: sand 36.2%, loam 37.9%, clay 25.9%, total N 0.121% and P (Olsen) 13 ppm. Exchangeable Ca, Mg and K, were 4,180, 641 and 142 ppm, respectively. Meteorological data were recorded trough SIAP automatic equipment controlled and validated by the Regional Hydrological and Geological Sector.

The experiment was carried out in rain fed conditions according to a Randomized Complete Block (RCB) design with five replicates. Sowing was performed on May 8. Sowing treatments (Fig. 1) included two single row spacing designs (18 and 60 cm) and one double row spacing design (18 + 60 cm). Seeding rate was 8.3 kg ha^{-1}. In order to attain a planting density of 25 plants m^{-2} (close to the optimal evaluated in previosus trials in the same area) seedlings were thinned at the two true leaf stage. Plots were 7 m long and the number of rows differed according to the different row spacing designs. Plots with 18 and 60 cm single row spacing had sixteen and four rows, respectively. The plots with double rows had eight rows: four pairs of rows spaced 18 cm apart and separated by 60 cm. Harvested rows were the middle 12 rows within the 18 cm plots and two rows in the both the 60 cm and the 18+60 cm plots, respectively. For the purpose of collecting the seed from an identical surface for all layouts of row spacing equivalent to 7.8 m^{-2}, the length of the rows in the test area corresponded to 3.5, 6.6 and 5.0 m, respectively for single rows, and 18 and 60 cm for double rows.
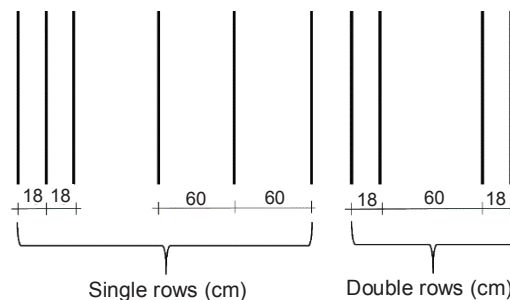

Figure 1. Layout of the row spacing utilized in the experiment.

A-61, a new breeding line of *Amaranthus cruentus* L., obtained by the University of Florence, was used. The line was obtained through a poly-cross between Mexican genotypes: PI-614882 as the female parent, and AMES-13729 and AMES-13734, respectively, as the male parents.

Fertilizer treatment before seeding was as follows: 76 kg ha^{-1} of N as ammonium nitrate, and 100 kg ha^{-1} of P_2O_5 as superphosphate. Plots were hand-weeded twice (38 and 52 Days After Emergence [DAE]) during the growth cycle. The incidence of sugar beet flea beetle (*Chaetocnema tibialis* (Illiger, 1807), was estimated at the two, four, and six true leaf stage. Immediately after the last estimation, the seedlings were treated with the insecticide, deltamethrine (50 mL 100 L water^{-1}).

Visual ground cover and the date of phenological plant stages, expressed as DAE, were recorded corresponding to emergence of the two, four, and six true leaf stages, respectively. Early panicle appearance, full panicle appearance, early flowering, milky maturation, waxy maturation and maturation at 75% were similarly recorded. For the maturation stage, seed consistency was taken in consideration together with complete filling (non-translucent endosperm). The final plant density was determined by counting plants in the sample area. The harvest was performed manually starting from September 18. Seed humidity at harvest was recorded on a 100 g sample for sampling area. After drying the seeds to a standard humidity of 12%, (airflow at 35 °C for 48 h), yield calculations were performed. Row spacing were considered as a factor with fixed effects in the ANOVA model. Differences between means were tested utilized Tukey test at $P \leq 0.05$, $P \leq 0.01$ or $P \leq 0.001$. COSTAT 6.45 software was used for the statistical analisys.

RESULTS AND DISCUSSION

Fig. 2 shows the climatic trends during the test period. Average minimum and maximum temperatures were 11.8 and 26.6 °C, respectively. Rainfall, that occurred in both the second and third ten days of April led to a delay in the sowing period compared to that considered optimal for the area. During the month of May, when the crop was at the two-four true leaf stage, temperatures ranged from 6.5 and 18.0 °C (below average monthly levels) with a rainfall of 127 mm. These environmental conditions led to a delay in plant development, thereby resulting in a delay of the physiological parameters characterizing the species in this phase of development. The last 10 days of July was characterized by both heavy and abundant rainfall, attaining a level of 233 mm of the total of 552 mm recorded in April-September period.
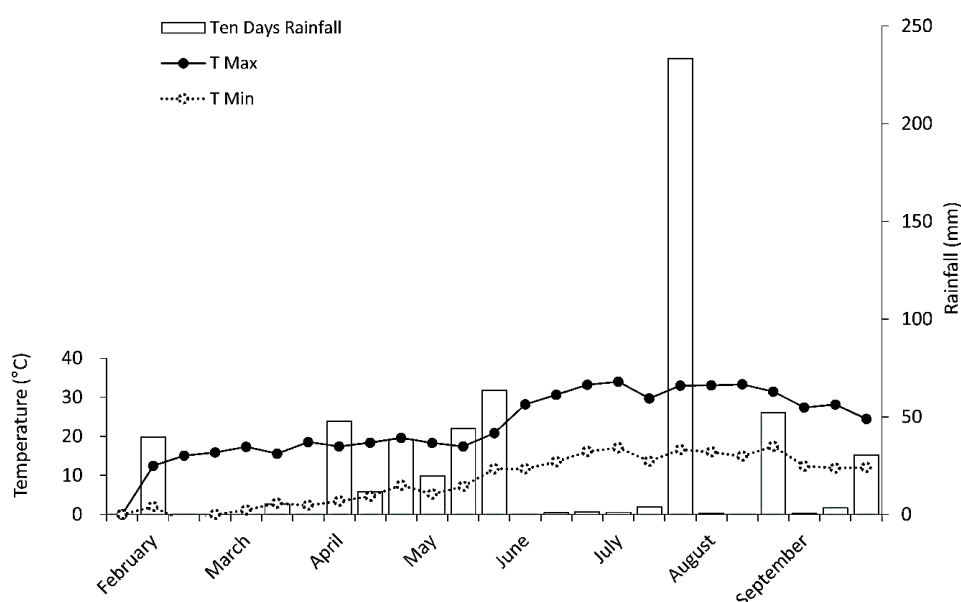

Figure 2. Temperature and rainfall recorded during the field experiment.

The analysis of variance (ANOVA) highlighted significant differences between the sowing designs, both in terms of different phenological phases and ground cover, respectively (Tables 1 and 2).

By analyzing in detail the phenological phases (Fig. 3), significant differences ($P \leq 0.05$) were reported from the beginning of panicle formation, i.e. coinciding with the initiation of the fastest growth phase in amaranth. This phase, characterized by physiology of the plant and the increase in average daily temperatures, occurred with a slight delay of about 5 d for all developmental phases in plants sown in single rows with a spacing of 18 cm. Evidently, intraspecific competition delayed development to full maturity in accordance with Henderson et al. (2000). The maturation of plants in the single row design of 18 cm, occurred at 127 DAE compared to 122 DAE for the remaining two experimental designs, respectively.

Table 1. Analysis of variance of the growth stages

Source of variation	DF	Four true leaves	Six true leaves	Ten true leaves	Early panicle	Panicle	Flowering	Milky maturity	Waxy maturity	Maturity
Blocks	4	0.40^{ns}	1.06^{ns}	1.07^{ns}	6.67^{ns}	6.67^{ns}	6.66^{ns}	6.40^{ns}	4.40^{ns}	3.73^{ns}
Row spacing	2	0.13^{ns}	1.73^{ns}	1.73^{ns}	32.93^{*}	32.93^{*}	32.93^{*}	19.1^{*}	21.73^{*}	48.53^{***}
Error	8	1.20	2.93	2.93	23.73	23.74	23.73	12.80	17.60	7.47
Total	14	1.73	5.74	5.74	63.33	63.33	63.34	38.4	43.73	59.73

ns: not significant; *: significant at $P \leq 0.05$; **: significant at $P \leq 0.01$; ***: significant at $P \leq 0.001$.

Table 2. Analysis of variance of the ground cover recorded at different growth stages

Source of variation	DF	Four true leaves	Six true leaves	Ten true leaves	Early panicle	Panicle	Flowering	Milky maturity	Waxy maturity	Maturity
Blocks	4	10.01^{ns}	56.67^{ns}	106.67^{ns}	93.33^{ns}	93.34^{ns}	6.66^{ns}	6.40^{ns}	4.40^{ns}	3.73^{ns}
Row Spacing	2	13.34^{ns}	2403.33^{***}	5063.34^{***}	2653.34^{***}	2653.33^{***}	32.93^{*}	19.1^{*}	21.73^{*}	48.53^{***}
Error	8	20.00	63.33	353.33	146.67	146.66	23.73	12.80	17.60	7.47
Total	14	43.33	2523.33	5523.32	2893.33	2893.34	63.34	38.4	43.73	59.73

ns: not significant; *: significant at $P \leq 0.05$; **: significant at $P \leq 0.01$; ***: significant at $P \leq 0.001$.
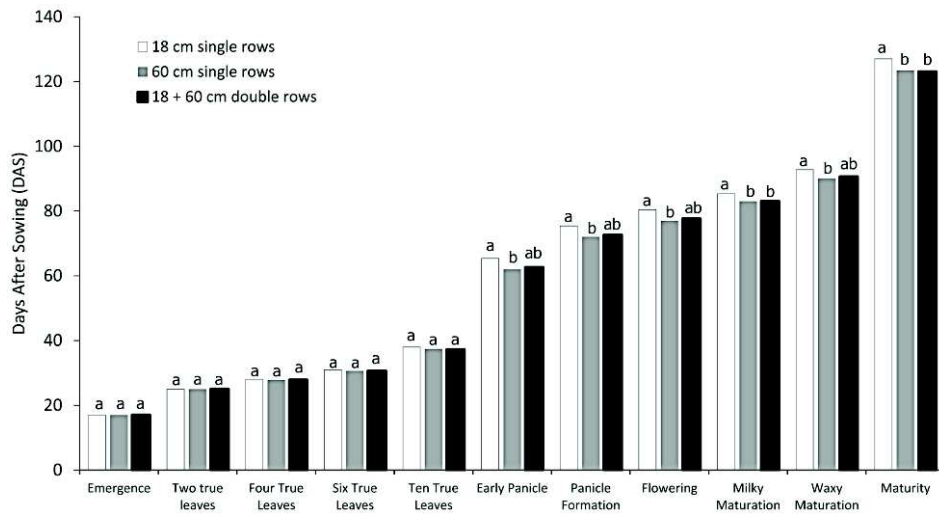
Figure 3. Date of the main phenological phases according to row spacing. Means within followed by same letter(s) are not different for $P \leq 0.05$ for the Turkey test.

The results pertaining to the ground cover (Fig. 4) permitted us to make some interesting observations. Starting from the six true leaf stage, prior to the rapid growth phase, there was a significant difference in ground cover between the different experimental row spacing designs. In the single row design of 60 and 18 cm row spacing, ground cover was 16% and 47% respectively. An intermediate coverage of 31% was evident in the double rows. In the subsequent growth phases of the crop, ground cover of the plants was similar in both the double row design and single rows with a spacing of 60 cm. In contrast, plants in the 18 cm row spaces exceeded an average of 28% ground cover up until maturity.
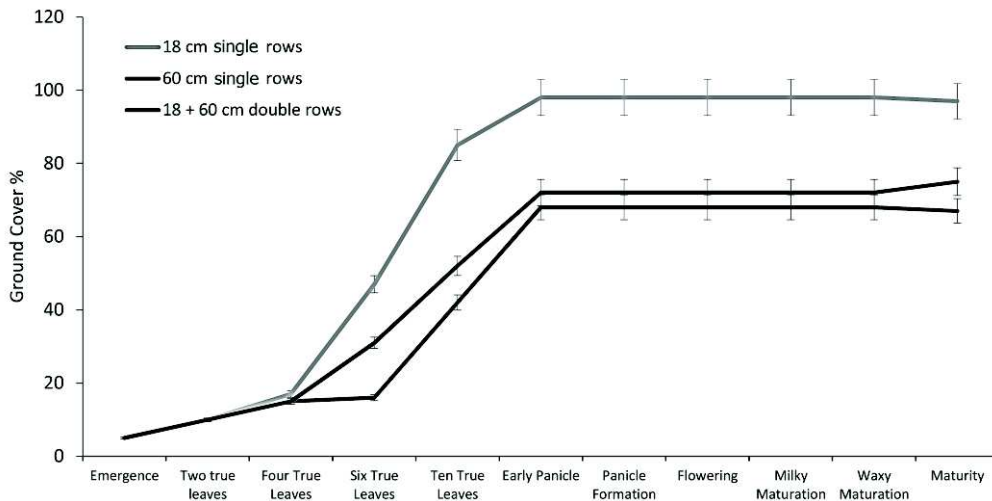

Figure 4. Ground cover according to the row spacing. Error bars represent the interval of the variability of the Tukey test. If the bars do not overlap, the difference between averages is significant at $P \leq 0.05$.

58

This behavior may be of particular importance to an early successful competition of amaranth against weeds, as was also reported by Gelinas & Seguin (2008). Prior to the ten true leaf stage, plants cultivated in single rows at 18 cm covered the ground early, thereby attaining a ground cover of 85% by the ten true leaf stage. This was significantly higher than the ground cover attained by plants in the double rows and single rows at 60 cm spacing, which was 52% and 42%, respectively. The present results corroborated previous findings (Gelinas & Seguin, 2008; Nurse et al., 2016), which also highlighted how reduced row spacing distances, despite the excellent competition exerted against weeds, manifested problems in the use of mechanical equipment as well as lodging where plant density was too high.

The different sowing designs did not influence diversification of sugar beet flea beetle attack (Table 3) which affected approximately 7–8% of the plants in the early stage of the crop (two true leaf stage) and then 50% at the six true leaf stage, at which point we intervened with deltamethrine treatment. The incidence rates of this insect are in accordance with that reported previously in different amaranth trials conducted in the same area (Casini & La Rocca, 2012; 2014; 2015). In Table 3, a significant effect on the height of amaranth at harvest was reported. Aside from the general reduction in crop development, attributable to environmental conditions, plants in the single rows of 18 cm were 20% shorter than those in the remaining two seeding designs. This effect is assumed to be due to the intraspecific competition mentioned previously, which reduced panicle development by 34%, compared to the remaining experimental designs.

Table 3. Incidence of sugar beet flea beetle, plant height, panicle length and plant density at harvest as affected by row spacing

Row Spacing	Sugar Beet Flea Beetle Incidence, %			Plant height, cm	Panicle length, cm	Plant density, n m^{-2}
	Two true leaves	Four true leaves	Six true leaves			
18 cm Single Rows	8 [a]	49 [a]	48 [a]	85.6 [b]	21.2 [b]	11.6 [b]
60 cm Single Rows	7 [a]	50 [a]	47 [a]	113.4 [a]	26,2 [b]	17.9 [a]
18+60 cm Double Rows	7 [a]	49 [a]	49 [a]	110.9 [a]	25.0 [ab]	17.3 [a]

Means followed by the same letter(s) are not different for $P \leq 0.05$ according to the Tukey test.

At harvest, a lower plant density of 11.6 plants m^{-2} in single row spacing of 18 cm was observed compared to the other two treatments reporting a density of 17.3–17.9 plants m^{-2}. The present results corroborated previous work by Myers (1996) using lower row spacing distances. That author attributed the low density to a 'self-thinning' put in place by amaranth when cultivated under conditions with more reduced row spacing distances.

The average seed moisture content at harvest (Fig. 4) was higher (23%) that the standard (12%) required for proper storage. The values obtained in the present study are similar to the averages observed in previous years in other amaranth trials carried out in the same period and area (Casini & La Rocca, 2012; 2014; 2015). However, seed humidity, derived from plants cultivated at a single row spacing of 18 cm (23.5%), was significantly higher than that recorded for the double rows. This may be attributable to the better distribution of plants in double rows with 60 cm spacing, which permitted improved air circulation.

Regarding yield, a seed production of 0.92 t ha^{-1} was obtained from plants in the double row design compared to the respective single row spacing designs of 18 and 60 cm, where yields were 0.85 and 0.70 t ha^{-1} respectively. Given that experiments using close double rows in amaranth were not found in the literature, it was not possible to make comparisons with the present results. A row spacing similar to the one used in this experiment, was adopted by Svirskis (2003) in Lithuania. This author reports that grain yield is higher in double rows spaced 50 cm apart at 100 cm compared to wider rows or arranged in four row strips 50 cm always spaced 100 cm apart.

Nonetheless, it has been noted that better yields have been obtained when row spacing was reduced (Jamriška 1998; Chaudhari et al.; 2009 and Olofintoye et al., 2011). In the present experiment, the double rows, though spaced by 60 cm, were very close (18 cm). Within the single row experiments, yield was higher at a row spacing of 18 cm than 60 cm.
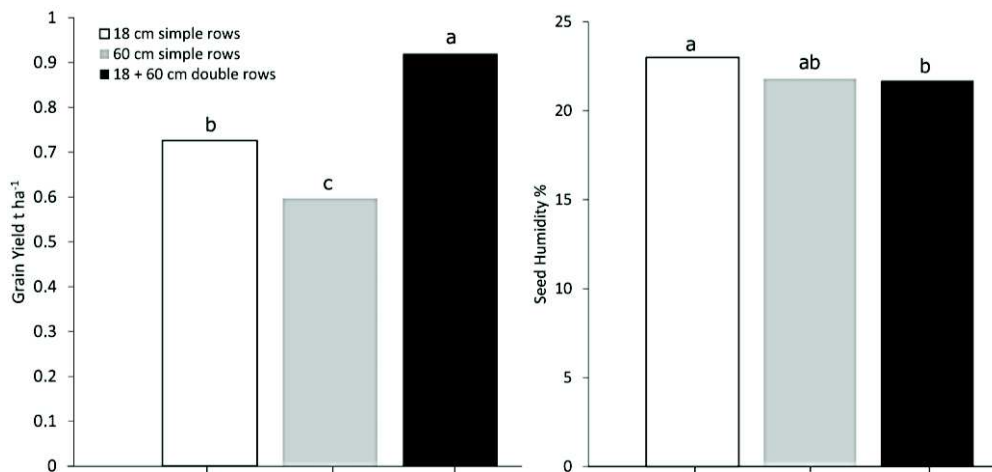

Figure 5. Seed humidity at harvest and grain yield production. Means followed by the same letter(s) are not different for $P \le 0.05$ according to Tukey test.

CONCLUSIONS

The results of the present trial, together with previous scientific reports, confirm amaranth as a species with considerable morphological plasticity. This feature enables the plant to manifest physiological and yield responses in function of row spacing despite environmental factors that also induce variable responses to different agronomic techniques (Gelinas & Seguin, 2008). Although yield is a major factor worthy of consideration, in order to be able to objectively assess the results of our research, additional aspects also require consideration. Firstly, the rapid ground cover manifested in the 18 cm single row spacing distance is interesting for a species like amaranth, characterized by a slow growth up until the six to ten true leaf stage. In the latter seeding design, a ground cover of 85% was attained early in the development, ensuring not only high competition against weeds, but also better potential soil erosion control (Weber, 1990). In some environments, this feature also facilitated faster dry-down and seed yield

(Wall, 1986). In fact, amaranth, even at the end of its reproductive cycle, has a tendency to dry very slowly. This behavior often forces to harvest seeds with high humidity (> 20%). In turn, additional costs must be incurred to dry the seeds to the standard 12%, considered suitable for safe storage.

The use of reduced row spacing distances requires careful selection in plant density. A high plant density may be associated with an excessive intraspecific competition and an increase of lodging (Myers, 1996; Henderson et al., 2000).

Currently no herbicides use on amaranth has been authorized; therefore, weed control must be performed mechanically. The use of very narrow row spacing greatly hinders mechanical interventions and, in order to overcome this difficulty, wider row distances must be employed (Sooby et al., 2005). Alternatively, it could be carry out a 'false seeding' to significantly reduce the weed seed stock before carrying out the amaranth sowing at proper date. However, the use of single row spacing distances of 60 cm resulted in a lower seed yield than that of the rows of 18 cm, while the use of double rows (18 + 60 cm) allowed the attainment of the best yield (0.92 t ha^{-1}).

Whilst single row spacing distances of 18 cm displayed a net of advantage against weeds, difficulties were encountered in the case of managing weeds by mechanical equipment. In this regard, the use of double rows appears to be a good compromise for farmers who can then also use equipment that can be used for other crops sown in rows (corn, sunflower, etc.). The use of double rows permitted taking advantage of a slight better ground cover than single rows, together with the possibility of mechanical intervention for the control of weeds, and importantly also produced a higher yield. The influence of climatic trend on both production and weed competition remains to be verified through additional experimentation.

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