Amaranth in southernmost latitudes: plant density under irrigation in Patagonia, Argentina[1]

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ABSTRACT

In Argentina there have been few evaluations of Andean pseudocereal plantings. This study explored the response of *Amaranthus cruentus* cv Mexicano to different plant densities under furrow irrigation in the lower valley of the river Negro, Patagonia, Argentina. The experimental design consisted of 3 blocks with randomized treatments (subplots), each one corresponding to different plant density. The treatments were sown in rows with spacing of 0.70 m (one row per ridge) and others with a spacing of 0.35 m (two rows per ridge). The plant densities evaluated were: 70,800 – 84,200 – 97,700 – 116,000 – 114,000 – 225,300 and 394,000 plants ha^{-1}. Different biometric variables and their components were measured: plant height, number of leaves, biomass and economic yield. The results suggest that the optimum plant density was 116,000 plants ha^{-1} with a row spacing of 0.70 m. This density produces an adequate plant stand from which to harvest optimal biological and economical yields. The contributions of this study demonstrated the potential of the *A. cruentus* crop in the lower valley of the river Negro, representing the southernmost study of plant density made for this pseudocereal in the world.

Keywords: crop geometry; biomass; economic yield; phenology; protein.

INTRODUCTION

The Andean pseudocereal world production has been growing worldwide due to its nutritional properties. Among them, the amaranth (*Amaranthus spp.*) stands out for its high percentage of protein (15-18%), lysine (5% on the dry basis) and the absence of gluten (Cassini & La Rocca, 2014). These characteristics indicate that amaranth is an important alternative crop for the future.

In order to increase the grain production, it is necessary to know how plant density influences the biometric parameters associated with yields. Leaf area, number of inflorescences, number of ramifications and stem diameter, among other things, are affected by plant density according to each species, cultivar and environment (Hass, 1983; Arellano, 2000). The high potential yield of the amaranth has been highlighted in various areas where it was cultivated. Alhough plant density has been the subject of many amaranth studies (Robinson, 1986; Henderson *et al*., 2000; Torres *et al*., 2006; Gimplinger *et al*., 2008; García Pereyra *et al*., 2009), information regarding this crop in Argentina is still limited.

Usually there is a range of densities for every cultivar and environment in which the yield is fairly constant, even though the yield per plant decreases. This is due to the fact that with each increment in density the loss per plant will be compensated by the increments in the number of plants per hectare. On the other hand, a lower or higher number of plants outside that range would result in lower yields. Similarly, the loss of plants and uneven seed emergence could cause a decrease in the yield per hectare which the number of plants at harvest would not be able to compensate. It has been suggested that the new genotypes are more tolerant to increments in density than in yield per plant (Tokatlidis & Koutroubas, 2004). The

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high potential yield of *Amaranthus* has been established not only in its area of origin but also in other areas where it has been introduced. The relationship between yield and plant density has been a matter of many studies in order to find the effect of plant density on yield and several biometric parameters (Robinson, 1986; Henderson *et al.*, 2000; Torres *et al.*, 2006; Gimplinger *et al.*, 2008; García Pereyra *et al.*, 2009).

The potential for amaranth cultivation in the extensive irrigated valleys of North Patagonia has not been considered previously. Thus, the aim of this study was to evaluate the effect of plant density and row distances on the biometric and production parameters in an irrigated crop of *Amaranthus cruentus* cv Mexicano, in the lower valley of the river Negro, Patagonia, Argentina.

MATERIALS AND METHODS

During the productive cycles 2011-2012 and 2012-2013, field experiments were conducted at the INTA Experimental Station in the lower valley of the river Negro (40°48' S, 63°05' W, 4 m above sea level). This area, with an irrigation system covering 24,000 hectares, is located in the province of Río Negro, Patagonia, Argentina. It has a semi-arid climate with the following average parameters during the trial period: temperature 19 ºC (± 3 ºC), evapotranspiration 576 mm (± 162 mm) and rainfall 31 mm (± 25 mm). January was the warmest month (23 ºC ± 1.5 ºC) and had the highest evapotranspiration values (181 mm ± 6 mm). March was the rainiest month (103 mm ± 11 mm). The initial physicochemical characteristics in the upper 50 cm of the experimental loam soil were: pH = 7.96; electrical conductivity = 0.50 mmhos cm^{-1}; organic matter = 2.38%; total nitrogen = 0.21%; P Olsen = 13.28 mg kg^{-1} (Zubillaga, 2017).

For both productive cycles studied the cultivar evaluated was *A. cruentus* cv Mexicano, sown by hand in a straight-line, at the end of spring (December 1st). The experimental plots had been left fallow for one year before starting the field experiments. Irrigation was applied before reaching the permanent-wilting point according to the curve of the soil-moisture retention, with a total lamina of 800 ± 50 mm. At sowing the experimental design comprised three 14 m^2 plots sown on one or both sides of the furrows. The plots were fertilized with 196 kg ha^{-1} of urea distributed as one dose when plants were 0.60 m high and a second dose at flowering.

The densities and space between furrows evaluated in this work were selected according to the previous experience by different authors (Henderson *et al.*, 2000; Malligawad & Patrol, 1999; García Pereyra *et al.*, 2009; Ramírez Vazquez *et al.*, 2011). For both productive cycles studied the experimental design included 3 blocks with 7 randomized treatments (subplots), each one corresponding to a different plant density. Some of these treatments were sown at a row spacing of 0.70 m (S: one row per ridge) and the others at 0.35 m (D: two rows per ridge). Following Zubillaga (2017), weeding and thinning-out of the crops was performed manually when the plants reached a height of 20-30 cm in order to get the appropriate density as shown in Table 1.

During the growth cycle, the following biometric parameters were measured on 10 plants per treatment and per subplot: maximum number of leaves (ML), maximum number of nodes (MN) and maximum number of ramifications (MR), with a weekly frequency of seven days. At the end of the growth cycle the selected plants in each treatment were harvested to measure the number of leaves at harvest (LH), ramifications at harvest (RH) foliar area at harvest (FAH), plant height (PH), panicle length (PL) and stem diameter (SD).

Thereafter, each plant was dried to constant weight at 60 ºC. Leaves (LW), stems (SW) and panicles including grains (PW) were weighed to obtain the total aerial plant biomass (aBW) on a dry basis. Each panicle was threshed with a suitably meshed sieve and grains were cleaned with a forced-air current. After that, the number of grains per panicle (NGp), dry weight (DWgp) and thousand grain weight (TGW) were recorded for each one. The inflorescence dry weight (IW) was calculated as the difference between the total panicle weight (PW) and the grain weight per panicle (DWgp).

Following Henderson *et al.* (1993), the phenological stages were recorded chronologically (days after seeding) with a weekly frequency of seven days: plant emergence (E), flowering (F) milky grain (MG) and physiological maturity (PM). The growing-degree days (GDD) for the total growth cycle (sowing to physiological maturity) were calculated according to McMaster & Wilhelm (1997).

The plants in the central furrows were harvested manually to study the biological (BY) and economic (EY) yields. The harvest index (HI) was calculated as the quotient between EY (kg of grain ha^{-1}) and BY (Kg biomass ha^{-1}).

Amaranth is characterized by its use as grain and fodder with high protein values (Barba de la Rosa, 2007; Seguin *et al.*, 2013). The chemical composition of different sections of the plant (leaves, stems and grains) was analyzed for the optimum density. The fiber content was determined as neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) using the Van Soest methodology (Van Soest *et al.*, 1991). The protein content (CP) was determined by the Kjeldahl method using factor 5.85 (AOAC, 1990). The ash (ASH) was determined by calcination at 550 °C (AOAC, 1990) and dry matter digestibility (DMD) by Rohweder equation (Rohweder *et al.*, 1978).

The statistical analysis was carried out with InfoStat software (Di Rienzo *et al*., 2016). A double ANOVA (years x treatments) was applied to each variable with a randomized block design in each year. The test did not detect any interaction between the years, so the analysis was performed by including all data from both years for each variable and treatment. The means comparisons were made with Fisher's minimum significant difference (DMS) at 5%.

RESULTS AND DISCUSSION

The density at harvest time (Table 1) was lower than the number of plants assigned to the corresponding treatments. The treatments S71-D86-S98 did not differ statistically ($p > 0.05$) from the initial planted density and harvest density. In the treatment with 97,700 plants ha^{-1} significant differences were found ($p > 0.001$) and the percentages of plant loss increased between 20-30%, which was due to the effect of intraspecific competition. Similar results for this crop were found by Henderson *et al*. (2000). In this regard, amaranth has the ability to compensate different levels of plant population due to its plastic morphology. As the plant population increased, the final population at harvest decreased, indicating plant mortally losses during the growing season. This self-thinning effect can be attributed to greater interplant competition for space, light, moisture and nutrients in larger populations (Henderson *et al*., 2000).

The row spacing and increase in plant density caused a significant decrease in all the biometric parameters evaluated at harvest time as can be seen in Table 2. The reduction in the plant height, the number of branches and the stem diameter with the increased population density was reported by Gimplinger *et al.*, (2008) and Cassini & La Rocca (2014).

Densities lower than 116,000 plants ha^{-1} did not cause any significant effects on LH, PH, and SD biometric parameters (Table 2).

The decrease in PH above 225,000 plants ha^{-1}, could be related to the decrease in MN due to interspecific competition. Similar results were reported by Henderson *et al.* (2000).

The increment in plant density induced a loss of FAH and a decrease in LH and RH, possibly associated with intraspecific competition. Higher densities generated greater shading between plants, which caused an early senescence of the leaves and branches. Plants can perceive the quality of light reflected from neighbors as an accurate predictor of future competition and respond morphologically even before they are shaded directly (Schmitt & Wulff 1993).

As observed in treatments S116 and D114 the effect of row space caused a decrease in the biometric variables, such as ML, MR, LH, RH, PL. Since there are no statistical differences in the number of plants, this effect can be attributed to the geometric distribution of plants that favors intraspecific competition. Similar results were found by Peiretti & Gesumaria (1991, 1998) in amaranth under different agroclimatic conditions.

Plant density was affected significantly BY and EY as shown in Table 3.

BY showed an increment directly related to the one seen in plant density. On the other hand, SW, PW, IW, LW and aBW showed a significant reduction when the density increased. Significant differences were found between aBW and its components (SW, PW, LW) at different row spacing and similar plant density (S116/D114) due to D114 being always lower than S116 (Table 3).

The increments in the amount of BY were directly associated with increments in the number of plants at harvest time. On the contrary, aBW and its components showed a decrease with each increase in plant density. The reduction of aBW would be compensated by the number of plants at higher densities which is reflected in the higher values of BY. The reduction of biomass per plant with the increase in density is due intraspecific competition which decreases the individual plant growth. Similar results were found by Putnam (1990); Malligawad & Patril (1999).

EY showed a tendency to increase with the plant density. However, there were no differences between S116

Table 1: Different seeding treatments tested for both productive cycles studied

Treatments	Initial density (plants ha^{-1})	Density at harvest time (plants ha^{-1})	Row spacing (m)
S71	71,500	70,800	0.70
D84	85,800	84,200	0.35
S98	110,000	97,700	0.70
S116*	143,000	116,000	0.70
D114*	143,000	114,000	0.35
D225	286,000	225,300	0.35
D394	572,000	394,000	0.35

*Plant number not statistically different by the Fisher LSD test ($p > 0.05$).

and the two higher densities (Table 3). The effect of geometric distribution at similar densities (S116 and D114) in BY, EY and its components were significantly different ($p < 0.01$) as can be seen in Table 3. In all cases S116 were higher than D114.

EY components, NGp, TGW and DWgp, decreased as the plant density increased. This clearly indicates that the higher grain yield per plant at lower plant densities could not compensate for the loss of EY due to the smaller number of plants per hectare. At lower plant densities (S71-D84-S98) the panicle showed a larger size, weight and number of grains. In this case, the plants had enough light and more leaf area (Table 2) to allow an increase in the production of photosynthates for their individual growth which led to a better grain development.

The maximum EY reached in this study was higher than the yields reported for the Pampas region of Argentina (Troiani *et al.*, 2004; Repollo *et al.*, 2010), as well as in other countries (see e.g. Gimplinger *et al.*, 2007), probably due to the availability of water for irrigation, differences in cultural practices and agro climatic conditions.

The optimum density was 116,000 plants ha^{-1} with a row spacing of 0.70 m. This density allowed a suitable stand of plants from planting to harvest. It also favored BY resulting in high EY, and therefore in optimum HI values. This row spacing between furrows could favor weed control and fertilization. At this density, competition between plants did not have any significant effect on their morphological structure. Similar results were found by Gimplinger *et al.* (2008).

Previously, Henderson *et al.* (2000) recommended an optimum density of 175,000 plants ha^{-1} and 0.75 m between the rows. In trials with a range of densities from 50,000 to 600,000 plants ha^{-1} some authors had found optimal density values between 180,000 and 210,000 plants ha^{-1} (Robinson, 1986); 375,000 plants ha^{-1} (Torres *et al.*, 2006); between

Table 2: Average values of biometric variables at the different plant densities tested

Variable	Plant densities and row spacing						
	S71	**D84**	**S98**	**S116**	**D114**	**D225**	**D394**
ML	47 a	46 ab	45b	43 c	41 d	39 e	36 f
MN	44 a	42 a	41 b	39 c	37 c	36 d	33 e
MR	14 a	12 bc	13 ab	11 c	8 d	7 d	4 e
LH	28 ab	27 ab	28 a	28 a	26 b	24 c	21 d
RH	7 a	5 b	6 b	5 b	4 c	2 d	0 e
FAH (cm^2)	1,750 a	1,689 b	1,699 b	1,606 c	1,586 c	1,459 d	885 e
PH (cm)	175 a	175 a	176 a	176 a	173 a	169 b	164 c
PL (cm)	46 a	45 a	45 a	43 b	40 c	36 d	28 e
SD (cm)	2.5 a	2.3 a	2.4 a	2.2 a	2.0 ab	1.7 bc	1.5 c

ML = maximum number of leaves; MN = maximum number of nodes; MR = maximum number of ramifications; LH = number of leaves at harvest; RH = ramifications at harvest; FAH = foliar area at harvest; PH = plant height; PL = panicle length; SD = stem diameter. Plant density abbreviations: see Table 1. Values of the same variable followed by the same letter are not statistically different by the Fisher LSD test ($p > 0.05$).

Table 3: Average values of biological and economic yield and its components at different plant densities tested

Variables	Plant densities						
	S71	**D86**	**S110**	**S143**	**D143**	**D286**	**D572**
BY (kg ha^{-1})	14,558 f	16,245 e	18,695 d	20,856 c	19,174 d	29,355 b	33,026 a
SW (g)	65 a	63 b	61 b	57 c	52 d	42 e	31 f
PW (g)	111 a	104 b	106 b	100 c	93 d	71 e	43 f
IW (g)	85 a	78 bc	80 b	75 c	70 d	57 e	35 f
LW (g)	29 a	27 b	25 c	23 d	22 e	18 f	10 g
aBW	206 a	193 b	192 b	180 c	167 d	130 e	84 f
EY (kg ha^{-1})	1,832 e	2,200 d	2,524 c	2,913 a	2,698 b	2,966 a	2,942 a
DWgp	26 a	26 a	26 a	25 b	24 c	13 d	7 e
TGW	0.87 a	0.87 a	0.86 b	0.86 b	0.85 c	0.85 c	0.79 d
NGp	29,659 ab	30,049 a	29,966 a	29,230 b	27,587 c	15,554 d	9,523 e
HI	0.13 a	0.14 a	0.14 a	0.14 a	0.14 a	0.10 b	0.09 b

BY = biological yields; DpH = plant density per ha at harvesting time; SW = stem dry weight; PW = panicle dry weight; IW = inflorescence dry weight; LW = leaf dry weight; aBW = aerial-plant–biomass dry weight; EY = economic yield; DWgp = dry weight of grains per panicle; TGW = thousand kernel weight; NGp = number of grains per panicle. Plant densities abbreviations: see Table 1. Values of the same variable followed by the same letter are not statistically different by the Fisher LSD test ($p > 0.05$).

50,000 and 140,000 plants ha^{-1} (Glimplinger *et al.*, 2008); and 125,000 plants ha^{-1} (García Pereyra *et al.*, 2009). The discrepancy in the different densities recommended by these authors is largely due to the environmental conditions where the crop develops and to cultural practices. The differences in yields found in this study in regard to those of these authors could be due to the causes mentioned above.

The total days between sowing and physiological maturity showed a tendency to increase with each increase in plant density. Densities S71, D86 and S98 did not show any significant statistical differences ($p > 0.05$) over the total length of the cycle (138 days). Densities with similar numbers of plants but a different geometry (S116 - D114) showed a length of 140 days. The highest densities (D225 - D394) reached 142 and 144 days respectively. The differences in the crop cycle are due to variations in the different lengths of the phenological stages (Figure 1).

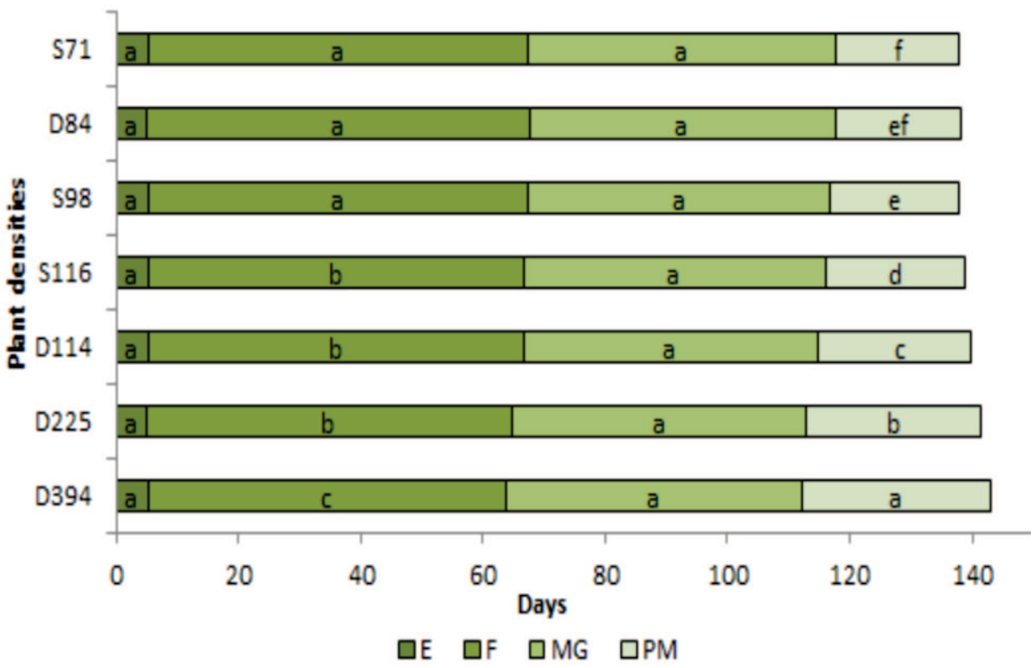
The E stage occurred in all treatments at the same time (5 days). The F period was reduced by an increase in plant density with a 3-day variation between the lowest and highest densities. MG did not show any statistically significant differences ($p > 0.05$) between treatments, the duration of this period was 50 ± 2 day. PM stages were extended with the increase of plant density with a 10-day variation between the lowest and highest densities.

The reduction in the F period with the density could be associated with the effect caused by intraspecific competition. Shade avoidance represents an important competitive strategy that plants possess and depends on multiple strategies. One of them is accelerated flowering (Smith & Whitelam, 1997; Callahan & Pigliucci, 2002; Botto & Smith, 2002). The signal to ûower would be earlier at higher plant densities because the ûowering time that would maximize the net photosynthetic rate is already earlier (Vermeulen, 2015).

When the growth cycle was expressed in GDD the results showed that the lapse between sowing and physiological maturity was 1689 GDD for the treatments S71, D84 and S98. Densities over than D114 showed an increment in GDD (1,696 GDD, 1,702 GDD, 1,716 GDD, respectively) until reaching 1,744 GDD in the D394 treatment.

The length of the amaranth growth cycle showed a tendency to increase according to the increase in plant density. The chronological days between sowing and physiological maturity at the lowest density were increased by 6 days at the highest density (55 GDD). This result is in opposition to other authors who found that the growth cycle either decreased in length, or did not change, as plant density increased (Putnam, 1990; Henderson *et al.*, 2000; Gimplinger *et al.*, 2008; Repollo *et al.*, 2010; Cassini & La Rocca, 2014).

A possible explanation for the extension of the crop cycle at higher densities may be given as a combination between the environmental conditions of the study site and the morphology of the panicles. From the month of April the temperature decreases (with probability of frost),



E = emergence; F = flowering; MG = milky grain; PM = physiological maturity. Plant densities abbreviations: see Table 1. Values of the same variable followed by the same letter are not statistically different by the Fisher LSD test ($p > 0.05$).

Figure 1: Duration of phenological stages at different plant densities tested.

Table 4: Chemical composition of stems, leaves, and grains for optimum plant density for both productive cycles studied

%	Grain	Leaves	Steam
NDF	17.01	43.78	63.06
ADF	7.5	15.43	45.64
ADL	4.29	3.42	5.31
ASH	2.42	23.16	15.21
CP	16.70	13.40	2.95
DMD	82.6	76.46	53.1

NDF: neutral detergent fiber, ADF: acid detergent fiber, ADL: acid detergent lignin, CP: crude protein, ASH: ashes, DMD: dry matter digestibility. Each value is the mean of N = 60. All values are expressed in dry matter. Values of the same variable followed by the same letter are not statistically different by the Fisher LSD test ($p > 0.05$).

the period of precipitation begins, the winds have lower intensity and evapotranspiration is reduced (Musi Saluj, 2018). The delay in the harvest time and the capacity of keeping the moisture for longer periods of time could be due to the conditions mentioned above in conjunction with the more compact structure of the panicles (small and short) at higher density of plants.

The extension of the crop cycle was a factor to be considered here, because the time of harvest overlaps with the onset of the rainy season. For this reason, early harvest could avoid an increase in panicle moisture, seed germination, loss by shattering, damage by birds or fungi and inclement weather.

The chemical composition of the different sections of the plant for the optimum density (S116) is shown in Table 4. Proximate analysis indicates that protein and fat are generally higher than in other common cereals (Singh & Singh, 2011; Pastor & Acanski, 2018).

The grain stands out for having a higher value of proteins and a lower fiber and ash content which generates greater digestibility.

Some authors have shown that the nutritive value of amaranth is equal to, or better than, commonly used forages. Its favorable composition as a ruminant feed is due to high crude protein (11.9%) and low content of lignin (4.5%) and it contributes to better digestibility (72.5%). Similar results were described by Sleugh *et al.* (2001) and Rezaei *et al.* (2009).

The optimum density (S116) reached the highest harvest index due to high yields in grain and biomass. Probably, the decrease in biomass per plant observed at higher densities would reduce forage quality due to the decreasing net assimilation, affecting the plant nutritional quality and the grain yield as mentioned by Yarnia (2010).

CONCLUSIONS

All the biometric variables decreased as the plant density increased. Intraspecific competition reduced the values of: plant height, panicle length, number of leaves and branches, weight and diameter of the stem. This was reflected in the lower individual biomass of the plants. This reduction in the source of photoassimilates affected the yield components (lower weight and number of grains per plant) and therefore the economic yield per plant. However, the greater number of plants compensated the yield in the grain per hectare, although the cost of seed and the length of the cycle of crop were increased.

The density of 116,000 plants ha^{-1} with a row spacing of 0.70 m can be considered as the optimum for the study area. This density allowed a suitable stand of plants from planting to harvest and favored the general development of the plants with high EY, optimal BY values and quality.

The agroclimatic conditions of the lower valley of Río Negro river are expressed as a potential area for the production of the amaranth crop in the Patagonia Argentina

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