Revista Eletrônica Interdisciplinar
Barra do Garças – MT, Brasil
Ano: 2025 Volume: 17 Número: 1
ISSN 1984-431X

CROPPING SYSTEMS, SOWING DATES, AND GROWTH OF CALENDULA CULTIVARS

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ABSTRACT

Increasing production of medicinal plants mainly depends on information about their cultivation characteristics. The objective of this study was to describe the growth of calendula cultivars in different dates and cropping systems. The experiment was setup in a randomized complete block design arranged in split plots consisted of three sowing dates [18 August/14 (D1), 22 October/14 (D2), and 08 April/15 (D3)]; two cultivars [Bonina Dobrada Sortida (BDS) and Bon Bon Yellow (BBY)]; and two cropping systems [direct seeding (DS) and seedling transplanting (ST)]. The cycle duration from emergence to harvesting ranged from 79 to 175 days in the three sowing dates. Cultivar BDS had the highest flowerhead height in all sowing dates and cropping systems. Direct seeding at D3 gave the highest means of plant height (20.30 cm) and leaf number (114.44 leaves). Cultivar BDS had higher plant height and leaf number in the three growing dates than cv. BBY. The highest mean diameter of flowerhead (7.03 cm) was recorded for cv. BDS. D3 gave the highest mean diameter of flowerhead (6.82 cm).

Keywords: *Calendula officinalis* L., seedling transplanting, direct seeding.

1. INTRODUCTION

Calendula (*Calendula officinalis* L.) or pot marigold has been used as a medicinal plant with anti-inflammatory and healing actions (Parente et al., 2009). It is also cultivated as ornamental because the yellow/orange variation of its flower colors. *Calendula officinalis* is a plant in the Asteraceae family, native to the Mediterranean region (Lorenzi; Matos, 2008). It is grown in southern Brazil because of the favorable climatic conditions of the region (Silveira et al., 2002). The use of medicinal plants in Brazil has increased as government health programs provided information on the benefit of their consumption, as well as the support to production and selection of materials to obtain quality products. Therefore, information on the requirements for production and commercialization of medicinal species is essential (Lourenzani et al., 2004). Among these

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ISSN 1984-431X

plants, calendula is listed as a species of medical interest by the Unified Health System (SUS), showing the importance of the research to improve cultivation techniques for the species (Santos et al., 2011).

The study of the interaction of factors such as sowing dates, cropping systems, and cultivars is needed for the understanding of growth and development of plants, which helps to improve cultural practices. Thus, studies are required with different sowing dates, cultivars (Duda et al., 2010), and different cropping systems such as direct seeding and seedling transplanting (Tivelli et al., 2009). There is little technical information available for growing species of small cultivated areas, especially medicinal plants, and studies must be performed.

Some studies on calendula phenology are found in the literature, however, with divergent results. In Chile, Berti et al. (2003) evaluated the effect of sowing season and different cultivars of calendula on yield of dry flowerheads and reported the highest yield for sowing in July (1300 kg of dry flowers ha^{-1}) in the first year, while in the second year, the highest yields were obtained in October (4042 kg ha^{-1}). Koefender et al. (2008) estimated phyllochron in calendula grown at different sowing dates and found influence of sowing dates on the number of leaves on the main stem and on the first lateral stem. Therefore, the objective of this study was to evaluate the growth of two calendula cultivars under different sowing dates and two cropping systems, direct seeding and seedling transplanting.

2. MATERIAL AND METHODS

The experiment was conducted in the field of the Agronomy Department of the Federal University of Santa Maria – UFSM, in the municipality of Santa Maria, RS, (29º43'23"S, 53º43'15"W, 95m altitude). The area is located in the Central Depression Region of Rio Grande do Sul and the climate is Cfa type (subtropical humid), according to Köppen classification (ALVARES et al., 2014). The chemical analysis of soil showed the following results: pH in water (1:1) = 5.8; organic matter = 3.2%; P = 12.6 mg dm^{-3}; K = 112 mg dm^{-3}; Ca = 99 mmolc dm^{-3}; Mg = 36 mmolc dm^{-3}; H + Al = 55mmolc dm^{-3}; Base Sum = 137.86 mmolc dm^{-3}; Effective CEC = 138 mmolc dm^{-3}; Base Saturation = 71.5%.

The experiment was setup in a randomized complete block design with five replications, in split plots in a trifactorial arrangement with 3 sowing dates [18 August (D1), 22 October (D2), and 08 April (D3)]; two cultivars [Bonina Dobrada Sortida (BDS) and Bon Bon Yellow (BBY)]; and two cropping systems [direct sowing (DS) and seedling transplanting (ST)]. Sowing dates were considered as the main plot and the combination of cultivars and cropping systems as the subplots, totaling twelve treatments.

ISSN 1984-431X

The following treatments were tested: T1: August, cultivar Bonina Dobrada Sortida, direct seeding; T2: August, cultivar Bonina Dobrada Sortida, seedling transplanting; T3: August, cultivar Bon Bon Yellow, direct seeding; T4: August, cultivar Bon Bon Yellow, seedling transplanting; T5: October, cultivar Bonina Dobrada Sortida, direct seeding; T6: October, cultivar Bonina Dobrada Sortida, seedling transplanting; T7: October, cultivar Bon Bon Yellow, direct seeding; T8: October, cultivar Bon Bon Yellow, seedling transplanting; T9: April, cultivar Bonina Dobrada Sortida, direct seeding; T10: April, cultivar Bonina Dobrada Sortida, seedling transplanting; T11: April, cultivar Bon Bon Yellow, direct seeding; T12: April, cultivar Bon Bon Yellow, seedling transplanting.

The blocks consisted of four flowerbeds measuring 2.25 m in length and 1 m in width and each flowerbed received a treatment. In direct seeding, 2-3 seeds of cultivars were seeded per hole at 2-3 cm depth spaced 0.3 m x 0.3 m between plants and 0.2 m x 0.2 m between rows. The net plot consisted of ten randomly selected plants after emergence and establishment of plants. Weed control was carried out by hoeing and irrigation by sprinkling. Pest and disease monitoring was performed, but control was not necessary.

In the seedling transplanting system, 2-3 seeds were sown per cell in 128-cell expanded polystyrene trays filled with commercial Mec Plant® substrate. After sowing, the trays were kept in a plastic-covered greenhouse with sprinkler irrigation timer programmed for three watering shifts daily, at 9:00 a.m., 1:00 p.m., and 4:00 p.m., of one minute each. Plantlets were thinned to one plant per cell, in both cropping systems, one week after the emergence. Seedlings with 4 to 6 leaves and more than 2.5 cm in height were transplanted to the field.

Fertilization was carried out according to the soil analysis and the recommendations of the Brazilian Soil Science Society - Soil Chemistry and Soil Fertility Commission of the Southern Regional Nucleus (SBCS, 2004) for cultivation of *Calendula officinalis* L. At 10 days after sowing and transplanting, 200 kg ha^{-1} of the commercial formula 5-20-20 (N-P_2O_5-K_2O) was applied to soil. Seedlings received the start fertilization in trays, with two fertigations of one liter of the solution water plus 1.5 g of the fertilizer 12-00-45 Krista K per tray, at 7 and 28 days after sowing.

The following characteristics were evaluated: plant height (PH) expressed as cm and measured from the collar to the last expanded leaf of the main stem; flowerhead height (FH), of the fully open flowerhead in the main stem, using a millimeter ruler; number of expanded leaves (LN), greater than 2.5 cm in length; (FD), diameter of flowerhead in the main stem using a pachymeter; and dry mass of plants (DM), expressed as grams, after drying in a

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forced-air circulation oven at 60ºC to constant mass.

Climate data were provided by an automatic weather station located at approximately 50 m from the experimental site. The average daily air temperature was calculated for each sowing date and the cumulative rainfall was calculated by the sum of the hourly rainfall data.

The assumptions of error normality was tested by the Shapiro-Wilk test and the homogeneity of variances by the Bartlett test. Subsequently, the analysis of variance (ANOVA) was performed and means were compared by the Scott-Knott test, using the statistical program Sisvar® (FERREIRA, 2014) at 5% error probability.

3. RESULTS AND DISCUSSION

The experimental errors related to the characteristics FH, PH, and DM had normal distribution and homogeneous treatment variances. However, LN and FD did not meet the assumptions and we used the Box-Cox transformation procedure (BOX; COX, 1964). After the transformation, the experimental errors LN and FD met the normality assumption, but not the homogeneity. As the normality assumption was met, the analysis of variance was performed, because error heterogeneity should not be considered so rigorously (CONAGIN *et al.,* 1993; PIMENTEL-GOMES, 2009).

Plants of treatments 1 and 3 seeded at D1 started flowering on 11/13/2014, 87 days after sowing (DAS). However, treatments 2 and 4 transplanted to the field on November 13, 2014 started flowering at 52 DAT. The crop cycle ended at 123 DAS in T1 and T3 and at 88 DAT in T2 and T4. Silveira *et al.* (2002) seeded calendula in trays kept in greenhouse in July 2000 in the southern region of Brazil and found that flowering started at three months after sowing, corroborating with the results of D1 in this study.

The difference in days for the beginning of flowering of plants seeded at D2 in T5 and T7 compared with T6 and T8 was the highest. This result may be related to the rapid growth of plants in T5 and T7 because of the higher average air temperature after germination compared with the treatments of D1 (T1 and T3). In addition, it took a longer time to produce seedlings in T6 and T8 (47 days) compared with T2 and T4 (34 days). This delay explains the difference in the growth of plants in T5 and T7 in relation to T6 and T8. Plants of treatments T5 and T7 seeded at D2 started flowering at 74 DAS. In contrast, treatments 6 and 8 transplanted to the field on 11/26/14 started flowering at 49 DAT.

We observed, at D3, that there was a longer time period from emergence to flowering and flowering to maturation of seeds compared with dates D1 and D2. Plants of treatments 9 and 10 were sown on 08/04/15 and started flowering

ISSN 1984-431X

at 93 DAS. T11 and T12 were transplanted to the field on 05/15/15 and started flowering at 113 DAT. The harvests of seeds, which marked the end of the crop cycle, were performed at 175 DAS for T9 and T11 and at 138 DAT for T10 and T12.

In April 2004, Gazim et al. (2007) conducted a study with direct seeding of calendula in the field and found that the inflorescences were at their maximum development at three months after planting, which is in agreement with the results observed in D3 plants. Angelini *et al.* (1997) reported a great variation in time from emergence to beginning of flowering (17 to 59 days) and from emergence to seed maturation (98 to 150 days). In the present study, the period from emergence to seed maturation (T9 and T11) took 159 days and from transplanting to seed maturation (T10 and T12) at D3 took 138 days, which is consistent with the finding of Angelini *et al.* (1997).

The longest crop cycle in D3 can be explained by the drop in average air temperatures in the first two months of cultivation and by the wide variation of average daily temperatures, which also occurred from July to the end of the crop cycle. In England, Cromack and Smith (1998) found that excessive rainfall increased the vegetative and flowering times of calendula. The climatic conditions of the present study were atypical because it was a year with a very strong El Niño and with excessive rainfall.

The average air temperatures in D1, D2, and D3 were 20.92ºC, 23.21 oC, and 17.01ºC, respectively, with cumulative rainfall of 1001.2 mm, 840.9 mm, and 1208.6 mm. Studies have reported that the optimal temperature for germination of calendula varies between 18 and 25ºC (LUZ *et al.,* 2001; KOEFENDER *et al.,* 2009). At the time of sowing of the three planting seasons, the average temperature was within this range. After germination, during growth, calendula endures higher temperatures (LUZ *et al.,* 2001), which shows that crop development was not affected by the air temperature.

The analysis of variance of the characteristic height of flowerhead showed a significant triple interaction between the sowing dates, cultivars, and cropping systems. The unfolding of the cultivars was performed within the combination dates*systems, as well as systems within dates*cultivar (Table 1). The characteristics PH, LN, and DM showed significant double interaction between dates and systems and between dates and cultivars. The unfolding of cropping systems and cultivars was performed within the dates (Table 2). There was a significant effect of cultivars and sowing dates on FD.

ISSN 1984-431X

Table 1. Average heights for inflorescence diameter (AC) - in cm - of marigold cultivars at different times of sowing, and cropping systems.

		Cultivars	
Date	Cropping systems	BDS*	BBY
August	Sowing	37.60a	26.33b
	Seedling transplanting	39.76a	24.40b
October	Sowing	36.25a	23.93b
	Seedling transplanting	35.32a	24.31b
April	Sowing	29.67a	19.39b
	Seedling transplanting	26.84a	16.94b
		Cropping systems	
Date	Cultivars	Sowing	Seedling transplanting
August	BDS	37.60a	39.76a
	BBY	26.33a	24.40a
October	BDS	36.25a	35.32a
	BBY	23.93a	24.31a
April	BDS	29.67a	26.84b
	BBY	19.39a	16.94b

*Averages followed by the same letter in the line differ significantly from each other Scott-Knott, at the level of 5% probability of error).

Table 2. Averages of characters as plant height (cm), number of leaves per plant and dry mass (g plant^{-1}) for cultivation seasons, direct sowing (Sow.), seedling transplanting (Trans.), and cultivars of marigold).

	Cropping systems					
	Plant height		Number of leaves		Dry mass	
Date	Sow.	Trans.	Sow.	Trans.	Sow.	Trans.
August	26.08b	27.67a	76.33a	74.66a	30.35a	21.55b
October	23.79a	24.00a	51.04a	37.73a	19.64a	20.10a
April	20.30a	17.25b	114.44a	55.42b	43.91a	27.99b
	Cultivars					

ISSN 1984-431X

	Plant height		Number of leaves		Dry mass	
Date	BDS	BBY	BDS	BBY	BDS	BBY
August	31.82a	21.93b	88.00a	62.99b	36.45a	15.45b
October	28.07a	19.72b	60.52a	28.25b	27.73a	12.01b
April	21.50a	16.05b	107.42a	62.44b	51.61a	20.29b

Averages followed by the same letter in the line, evaluating the same caracteres, differ significantly from each other Scott-Knott, at the level of 5% probability of error.

Flowerhead height of cultivar BDS was greater than cultivar BBW in all dates*systems combinations. Information from the seed supplier shows that the commercial height of BDS varies from 25 cm to 40 cm and BBW of 30 cm. Therefore, to obtain plants with greater flowerhead height intended for cutting, one should choose the cultivar BDS, regardless of the sowing date or the cropping system (direct seeding or transplanting). Besides, the flowerhead height of both cultivars were not influenced by the cropping system at D1 and D2. However, at D3, the flowerhead height of the two cultivars was favored when cultivated in the system of direct seeding (Table 1).

There was interaction between the dates and cropping systems for PH, LN, and DM. The characteristic PH at D1 showed higher mean under the system seedling transplanting. On the other hand, there was no significant difference between the D2 means and, the direct seeding provided higher plant height at D3 (Table 2).

The lower accumulated precipitation (1001.2 mm) at D1 associated with higher average air temperature (20.92°C) favored plant growth (higher height) and consequent reduction in leaf number. At D3, the plants completed the cycle with higher accumulated rainfall and lower average temperature (17.01°C), which resulted in higher development (leaf number) and lower growth. Consequently, a greater photosynthetically active area gave rise to plants with higher dry mass (Table 2). PH means in the cropping systems varied between 26.08 cm and 27.67 cm at D1 and between 20.30 cm and 17.25 cm at D3.

The characteristic LN showed no significant difference between the cropping systems at dates D1 and D2. At D3, the direct seeding gave the highest mean (114.44 leaf plant^{-1}) (Table 2). The overall mean of leaf number at dates D3 and D1 was 84.93 leaf plant^{-1} and 75.49 leaf plant^{-1}, respectively, which were higher than the mean of D2, 44.38 leaf plant^{-1}. Koefender *et al.* (2008) estimated phyllochron in calendula grown under plastic-covered greenhouses and found that the total number of leaves in the main stem of plants sown in October was smaller than those sown in April.

ISSN 1984-431X

However, in the same study, Koefender *et al.* (2008) obtained a high coefficient of determination for the ratio between the number of leaves formed in the main stem and the accumulated thermal sum, indicating that air temperature is the main environmental factor that determined the production of leaves in calendula. In this study, there was no control of rainfall. Our findings show that the for mean leaf number at the sowing dates, there may have been other factors that influenced the leaf production, since no direct relation of average air temperature and leaf production was observed.

The characteristic dry mass showed no significant difference between cropping systems at D2. The direct sowing had the highest means at D1 and D3 (Table 2). Dry mass production is characteristic used to quantify plant growth (SERRA *et al.*, 2012), which indicates that direct seeding provided higher growth in relation to seedling transplanting.

Depending on the objective of growing the crop, the direct seeding can be advantageous as it produces plants with greater mass, height, and number of leaves. If the objective is compound extraction and production of phytotherapeutic drugs, in which the shoot is used, plants with higher dry mass will provide higher yield of raw material. On the other hand, seedling transplanting will depend mainly on availability of labor, infrastructure, and the time of land occupation. In this study, we report a reduction of 35, 48, and 37 days in the crop cycle of the treatments with seedling transplanting at the dates D1, D2, and D3, respectively, compared with the treatments with direct seeding. In an intensive land use system, information on the crop cycle length is crucial for decision-making on choosing the cropping system.

Cultivar BDS had higher means of PH, LN, and DM than cv. BBY, independently of the sowing date (Table 2). The production of greater number of leaves is important when the objective is extraction of the essential oil used by pharmaceutical and cosmetic industries. It is likely that the superiority of cultivar BDS is a genetic characteristic, since it was maintained over the sowing dates. Under adequate conditions of cultivation, collection, drying, and extraction of the volatile fraction, it is possible to obtain chemical constituents similar to those obtained in their natural habitat (GAZIM *et al.*, 2007).

The cultivars were significantly different for the characteristic FD, with cultivar BDS having higher mean (7.03 cm) than cultivar BBY (5.01 cm). The sowing dates also differed for the characteristic FD: the highest D3 mean of 6.82 cm differed from the intermediate D1 mean of 5.96 cm and from the lowest D2 mean of 5.28 cm.

In their investigation into field productivity of calendula inflorescences under supplemental irrigation depths in the region of Oeste Paulista, Marques *et al.* (2011) shows that

ISSN 1984-431X

supplementary irrigation applied at the pre-flowering stage induced the maximum flowering, suggesting that the water supply is directly related to the quantity of flowerheads produced. In the present study, the highest accumulated rainfall occurred at D3, with large water volumes at the pre-flowering stage. Thus, in order to produce more flowerheads with larger diameter, cultivation during seasons of higher accumulated rainfall should be prioritized for higher productivity.

4. CONCLUSION

Therefore, we concluded that the time from emergence to seed maturation varied according to the sowing dates, and the longest cycle, flowering and growth periods, occurred at the sowing date D3. Cultivar BDS showed higher plant height, greater number of leaves, and greater flowerhead diameter at the three sowing dates. We recommend direct sowing as the cropping system because it provides higher flowerheads and greater number of leaves at the sowing date D3, which also provided flowerheads with greater mean diameter.

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