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Impact of row spacing, planting pattern, and cover crop on controlling weeds and improving antioxidant defense system, osmolyte accumulation, and plant performance in maize

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Abstract

Objective: This study investigated the effects of row spacing, planting pattern, and weed control methods on maize grain yield, weed control, antioxidant defense system, and osmolyte accumulation.

Methods: To conduct the experiment, the maize cultivar TWC647 was planted in the row spacings of 75 cm and 65 cm, using two planting patterns, conventional single-row (CSR) and zigzag double-row (ZDR). Also, five different weed control methods were implemented, including a weedy check throughout the growing season, *Trifolium alexandrinum* L., *Secale cereale* L., *Vicia villosa* L., and the herbicide MaisTer Power OD® 42.5% (containing foramsulfuron and idosulfuron). Then, the protein content, H_2O_2, total phenols, total soluble proteins, proline, superoxide dismutase (SOD), peroxidase (POX), catalase (CAT), and malondialdehyde (MDA), were measured.

Results: The biomass of cover crops was significantly affected by row spacing, planting pattern, cover crop, and their two-way interactions. The highest cover crop biomass was obtained for the 75 cm row spacing with the CSR planting pattern. The rye cover crop showed the highest biomass in both row spacing and the CSR planting pattern. The distribution of grass weed species was relatively even throughout the experimental site. The lowest weed biomass was obtained in rye and vetch with the 65 cm row spacing and also under both CSR and ZDR planting patterns. Maize grain yield was significantly affected by the row spacing × cover crop interaction, with maize alongside the rye cover crop producing the highest grain yield per hectare in the 65-cm row spacing, significantly higher than in the 75-cm row spacing. MDA was higher at the 65 cm row spacing and under CSR planting pattern than at the 75 cm row spacing and ZDR planting pattern. The lowest and the highest MDA among the weed-control methods were observed for the rye cover crop and the herbicide MaisTer. H_2O_2 was also higher

in the 65 cm row spacing. The highest H_2O_2 content of maize leaves was obtained for the herbicide MaisTer under CSR. Proline, total proteins, and total phenols were also greater at the 65 cm row spacing.
Conclusion: The narrow row spacing (65 cm) with rye inter-row cover crop reduced weed biomass compared to wider row spacing (75 cm). The 65 cm row spacing also resulted in higher grain yield of maize when rye cover crop was planted among the maize rows. The 65 cm row spacing also exhibited higher total proteins, total phenols, proline, MDA, and H2O2 content. However, the rye cover crop decreased H_2O_2 and MDA compared to the herbicide and weedy check.

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Introduction

Maize (*Zea mays* L.) is known for its remarkable grain yield per unit area, surpassing that of rice and wheat. Increasing maize productivity has been a focal point in numerous studies (Ren *et al.* 2020). These investigations have unveiled that genetic factors contribute to 50-70% of maize yield improvement, while agronomic practices account for 30-50% (Lee and Tollenaar 2007). Among the various agronomic factors, plant population, row spacing, and soil fertility significantly influence maize grain yield (Tollenaar and Lee 2002; Raymond *et al.* 2009). According to Andrade *et al.* (2002) and Robles *et al.* (2012), narrow-row planting enhances light absorption during the pollination phase in maize, which leads to improved grain yield. Using narrow rows of 65 cm increased maize performance from 2.7 to 10% (Nelson *et al.* 2015; Widdicombe and Thelen 2002). However, Yadete Urge (2022) reported varying results regarding the yield advantage of narrow rows (less than 30 inches) compared to wider rows (more than 30 inches).

However, the literature presents varying results regarding the yield advantage of narrow rows (less than 30 inches) compared to wider rows (more than 30 inches) (Yadete Urge 2022). Using narrow rows of 65 cm increased 2.7 to 10% maize performance Widdicombe and Thelen 2002).

The photosynthetic performance of a crop is closely linked to the conditions surrounding the canopy, namely the microenvironment, which includes CO_2 level, temperature, light, and humidity (Zhang *et al.* 2021). Proper row direction and spacing have a positive impact on the microenvironment within the canopy. Research indicates that photosynthetic production is significantly higher in rows with 70-cm and 50-cm spacing compared to rows with 65-cm and 60-cm spacing (Ge *et al.* 2022).

Additionally, employing double-planting with wide rows helps to delay the aging process in the middle and late stages of crop growth (Wei *et al.* 2012). Within-row spacing of 15 cm compared with 18 cm has been associated with several positive effects in plants, including a slower chlorophyll degradation and leaf aging, a reduction in malondialdehyde (MDA) level, an increase in antioxidant enzyme activity, and higher grain weight per spike (Zheng Feina *et al.* 2020).

It's widely acknowledged that appropriate row spacing effectively mitigates the activities of catalase (CAT), peroxidase (POX), superoxide dismutase (SOD), and non-structural carbohydrates (Ge *et al.* 2022). Additionally, it helps reduce lipid peroxidation of cellular membranes and inhibits the production of MDA and premature aging (Tian *et al.* 2019). The planting pattern plays a significant role in shaping the canopy structure and affecting the efficiency of radiation utilization and light accessibility (Chapepa *et al.* 2020). The choice of planting pattern is heavily influenced by both row spacing and plant density. When plants are grown in a square planting pattern, the optimal canopy structure, enhanced ventilation, and effective light interception result in robust plant growth (Tang *et al.* 2019; Ibrahim *et al.* 2022). Planting patterns modulate leaf morphogenesis responses, subsequently shaping the spatial arrangement of leaves to minimize mutual shading and optimize light capture (Xue *et al.* 2023). Upright leaves, which facilitate reduced light attenuation within the canopy, can lead to increased yield when compared to flat leaves (Digrado and Ainsworth 2023). Also, wide row spacing promotes a more uniform distribution of light within the canopy, thereby improving ventilation, light utilization, and the photosynthetic efficiency of the middle and lower leaves (Ibrahim *et al.* 2022).

Cover crops between maize rows improve water and soil quality by boosting soil organic matter and nutrient recycling, reducing nitrogen and phosphorus runoff, and suppressing weeds (Adetunji *et al.* 2020). However, their use is limited in some areas, partly due to concerns over potential negative impacts on maize yields. Understanding these effects is essential for developing strategies to mitigate risks and enhance the benefits of cover crops in local agriculture.

In modern crop production, herbicides are crucial for weed control, supporting yield and economic benefits (Chauhan 2020). However, they can cause abiotic stress and phytotoxicity, affecting plant height, shoot dry weight, leaf area index, and altering metabolism through oxidative stress (Wang *et al.* 2022). MaisTer Power, a post-emergence herbicide safe for crops, targets weeds' aceto-hydroxy-acid-synthase enzyme with a mix of active ingredients (El-Sayed *et al.* 2021). Although it is effective in controlling grasses and broad-leaved weeds, its use may initially suppress growth and result in chlorosis, necrosis of shoots, and ultimately, plant mortality.

Focusing on the TWC647 cultivar, this study aimed to examine how row spacing, plant density, and cover crop affect grain yield and biochemical traits, assessing whether narrower spacing reduces competition and increases yield in high-density setups.

Materials and Methods

The experiment was conducted at the Agricultural and Natural Resources Research Center of Moghan, Iran, during the 2022 season. The average monthly air temperatures and precipitation exhibited fluctuations in the 2022 growing season (Table 1). The experiment employed a split-plot factorial design based on a randomized complete block design with three replications. The main-plot factor consisted of two row spacings (75 cm and 65 cm). The subplot factor was a factorial combination of two planting patterns [(conventional single row (CSR) and zigzag double row (ZDR)] with five different weed control methods as a) weedy checks throughout the growing season, b, c, and d) the use of vetch (*Vicia villosa* L.), clover (*Trifolium alexandrinum* L.), and rye (*Secale cereale* L.), respectively, and e) spraying with MaisTer® herbicide at rates of 1.5 L/ha when the maize plants had 3-4 leaves.

Table 1. The average monthly air temperatures and precipitation in the experimental site.

Year	Month	Average of sunshine hours (h day^{-1})	Average of evaporation (mm day^{-1})	Humidity (%)	Rainfall (mm)	Average of temperature (◦C)	Max temperature (◦C)	Min temperature (◦C)
2022	Jan	4.2	0.0	78.3	10.3	3.7	9.1	-1.7
	Feb	4.5	0.0	73.8	7.3	4.0	9.3	-1.6
	Mar	5.5	0.0	64.6	1.3	11.3	18.2	4.3
	Apr	6.0	4.5	64.5	8.1	12.6	19.4	5.7
	May	6.3	5.0	70.2	12.4	17.8	24.1	11.6
	Jun	10.3	8.9	60.3	23.1	23.9	31.5	16.3
	Jul	10.2	9.8	54.6	4.8	27.5	34.3	20.7
	Aug	9.2	7.9	64.5	8.6	27.3	34.1	20.5
	Sep	7.8	5.3	65.3	0.0	24.1	31.2	17.0
	Oct	5.2	2.9	67.7	6.8	20.3	25.7	14.9
	Nov	5.3	1.1	77.8	27.4	12.6	18.0	7.2
	Dec	0.9	0.4	87.1	15.9	7.2	9.8	4.6

Maize seeds were planted in a field prepared by disking followed by cultivator tillage. Furrows were created at two different spacings, 65 cm and 75 cm. The experimental units consisted of four rows, each 5 meters in length. A medium-maturing maize variety, TWC 674, commonly grown in Iran, was planted during the 2022 growing season on May 7. A precision plot planter with adjustable row spacing was used for planting the maize seeds. Both maize and cover crops were sown simultaneously. Cover crop seeds were manually broadcast between the rows at specific rates: 160 kg/ha for rye, 45 kg/ha for hairy vetch, and 30 kg/ha for clover. For the maize crop, fertilizers were applied at 250 kg/ha of urea, 250 kg/ha of P_2O_5 (superphosphate), and 100 kg/ha of KCl. These fertilizers were applied in furrows located alongside and below the sowing furrows. Initially, only 30% of the total nitrogen (N) was used, combined with the P_2O_5 and K_2O, which served as the base fertilizers. The remaining nitrogen (N) fertilizer was used as a top dressing. The effective control measures were implemented for irrigation, diseases, and insect pests to ensure that no external factors limited plant growth.

Determination of hydrogen peroxide (H_2O_2)

The measurement of H_2O_2 content followed the procedure described by Talaat *et al.* (2015). For the leaf tissues, a 50 mM buffer with a pH adjusted to 6.8 was used, employing 3.0 mL of phosphate buffer per 0.5 g of leaf material. The homogenate was then centrifuged at 6000 × g for 25 minutes. Subsequently, 0.1% titanium chloride in sulfuric acid (20% v/v) was added to the extract, and the mixture was centrifuged again at 6000 × g for 15 minutes. The H_2O_2 content was quantified using spectrophotometry (UVmini-1240, Shimadzu Corporation, Japan) at a wavelength of 410 nm. A standard curve was generated by plotting absorbance values against the known H_2O_2 concentrations.

Determination of lipid peroxidation

MDA equivalent was used as the indicator of lipid oxidative damage. To do this, fresh leaves (0.5 g) were homogenized in 80% ethanol. Subsequently, centrifugation was carried out at 4 °C for 10 minutes at 3000 × g. The resulting pellet was subjected to a two-fold extraction with 80% ethanol and then transferred to a new test tube. A solution composed of butylated hydroxytoluene (0.01%), thiobarbituric acid (0.65%), and trichloroacetic acid (20%) was prepared and added in an equal volume to the supernatant from the previous step. This mixture was incubated at 95 °C for 25 minutes and then cooled to room temperature. Spectrophotometric measurement was conducted at a wavelength of 532 nm, and the obtained values were used in the MDA calculation formula (Noreen and Ashraf 2009). To ensure accuracy, measurements were obtained from the newest fully grown leaves of three distinct plants.

Measuring protein content and antioxidant enzyme activity

The Crude leaf extracts were prepared using a Tris–HCl extraction buffer with a ratio of 1:2 (W/V), based on the Soltis and Soltis (1990) protocol, later modified by Moharramnejad and Valizadeh (2019). The extracts were then centrifuged at 4 °C for 10 minutes at 10,000g. Enzyme extracts were absorbed directly using filter paper. The prepared extracts were subjected to electrophoresis on a horizontal slab polyacrylamide gel (7.5%). This process was conducted using an electrode buffer composed of Tris–Borate-EDTA (TBE; pH = 8.8) for 4 h at 4 °C. After electrophoresis, two slices were excised from the slab gels and subsequently stained to assess enzyme activities. The activities of SOD, POX, and CAT were assessed using the staining procedures adapted from Soltis and Soltis (1990) and later modified by Moharramnejad *et al.* (2019). Subsequently, the stained gels were fixed and scanned at a suitable range of wavelengths. For each isozyme activity, the calculation of optical density multiplied by the area was carried out using image analysis software called MCID. Additionally, the total protein content associated with the enzyme extracts was quantified using the Bradford (1976) method.

Determination of total phenols

For determining total phenolics, 50 mg of fresh leaf tissue was ground well in 80% acetone, followed by centrifugation for 10 minutes at 10,000 g. Then, 2 mL of water and 1 mL of Folin–Ciocalteau's phenol reagent were added to dilute 100 µL of the supernatant. The resulting mixture underwent intense shaking. The process then involved adding a 5 mL solution of sodium carbonate (20%) and adjusting the volume to 10 mL with distilled water. After thorough mixing, the absorbance was measured at a wavelength of 750 nm, following the method outlined by Noreen and Ashraf (2009). Subsequent calculations were carried out to express the final data as milligrams per gram (mg/g) of fresh leaf material.

Determination of proline

The process involved treating dried ground leaves with a toluene–water mixture containing 0.5% toluene (5 mL per 0.1 g of plant material). Following 24 h of continuous shaking at 25 °C, the extract was subsequently filtered, and the volume was adjusted to 100 mL. To 1 mL of the solution, 1 mL of

2 N HCl was added. Additionally, a 0.1 mL solution of potassium triiodide was added to 0.5 mL of the solution obtained from the previous extraction step. Following 90 minutes of shaking in an ice bath, 2 mL of ice-cooled water and 4 mL of 1,2-dichloroethane were added. Upon stirring, two phases separated within the mixture. The isolated lower phase underwent measurement of the optical density at 365 nm. This step was employed to determine the proline content, following the methodology outlined by Bates *et al.* (1973). To prepare the extract from fresh leaves, a mixture of sulphosalicylic acid, ninhydrin, glacial acetic acid, and acetic acid solutions was used. Following the incubation of the samples at 100 °C, 5 mL of toluene was introduced. The toluene phase was then transferred to a new container, and its optical density was measured at a wavelength of 528 nm. To determine the proline content, a standard curve was established by assessing the absorbance of various proline concentrations from Sigma, USA.

Statistical analysis

The analysis of variance was conducted to assess the significance of main effects and their interactions. The means were compared using Duncan's multiple range method at 5% probability level.

Results and Discussion

The analysis of variance of the data showed significant effects of row spacing, cover crop, and planting pattern on the biomass of cover crops. Furthermore, the interaction of row spacing × planting pattern, row spacing × cover crop, and planting pattern × cover crop was also significant concerning the biomass of the cover crops (Table 2). Mean cover crop biomass was higher in the 75 cm row spacing compared to the 65 cm row spacing (28.14 g m^{-2} and 25.05 g m^{-2}, respectively), and mean cover crop biomass was higher in the CSR planting pattern compared to the ZDR planting pattern (27.96 g m^{-2} and 25.21 g m^{-2}, respectively). The lowest and the highest cover crop biomasses were achieved for the 65 cm row spacing under ZDR planting pattern (23.66 g.m^{-2}) and 75 cm row spacing under CSR pattern (29.50 g.m^{-2}), respectively (Figure 1). The rye cover crop displayed the highest biomass (50.96 g.m^{-2}) in 75 cm row spacing (Table 3). Rye also displayed the highest biomass (50.48 g m^{-2}) in the CSR planting pattern (Table 4).

The weed biomass was affected by the interactions of row spacing × cover crop and planting pattern × cover crop (Table 2). The lowest weed biomass was achieved in rye (10.40 g.m^{-2}) and vetch (11.73 g.m^{-2}) with the 65 cm row spacing (Table 3). Based on the planting pattern and cover crop interaction, the lowest weed biomass was recorded in rye, vetch, and clover under both the CSR and

Table 2. Analysis of variance for the maize traits under different row spacings, planting patterns, and cover crops.

SOV	df	Mean Squares						
		Cover crop biomass	Weed biomass	Grain yield	H_2O_2	Total phenols	Proline	Total protein
Replication (R)	2	31.40^{ns}	26.69^{ns}	0.69^{ns}	119.08**	0.006*	10.46**	1.32E-4*
Row spacing (S)	1	4382.73**	2290.85**	0.03^{ns}	6475.88**	4.78**	185.79**	4.145**
R × S	2	37.44	7.67	6.72	0.80	0.052	0.07	1.67E-6
Planting pattern (P)	1	1036.67**	1813.42**	0.01^{ns}	1337.65**	0.169**	276.15**	0.006**
Cover crop (C)	4	1719.41**	114993.90**	0.74^{ns}	7541.27**	0.079**	90.03**	0.004**
P × C	4	36.49**	39.65**	0.64^{ns}	82.89**	0.013**	1.84^{ns}	2.64E-4**
S × P	1	155.52**	0.03^{ns}	1.27^{ns}	9.02^{ns}	0.001^{ns}	1.86^{ns}	1.50E-5^{ns}
S × C	4	119.82**	413.35**	3.73*	9.85^{ns}	0.001^{ns}	0.61^{ns}	4.41E-5^{ns}
S × P × C	4	6.52^{ns}	2.49^{ns}	0.30^{ns}	0.56^{ns}	1.12E-4^{ns}	0.01^{ns}	1.91E-5^{ns}
Error	36	6.46	13.82	1.74	9.86	0.002	1.04	2.78E-5
CV (%)		9.56	13.85	19.13	2.57	7.01	4.08	2.328

Table 2 continued

SOV	df	Mean Squares						
		SOD_1	SOD_2	SOD_3	POX_1	POX_2	CAT	MDA
Replication (R)	2	4622.96**	14146.47**	2559.61**	105.48**	1773.30**	3637.48**	0.11^{ns}
Row spacing (S)	1	412742.62**	470606.07**	651585.53**	24064.84**	31457.27**	74940.59**	589.07**
R × S	2	31.17	95.39	17.27	0.71	11.94	24.53	0.001
Planting pattern (P)	1	65583.11**	161032.73**	85685.80**	17064.34**	28239.51**	87201.79**	756.86**
Cover crop (C)	4	165790.27**	229405.45**	317189.08**	14638.84**	19603.24**	158741.94**	2489.92**
P × C	4	4020.60**	9856.10**	3391.81**	814.83**	550.33*	981.67^{ns}	4.79^{ns}
S × P	1	442.17^{ns}	1085.71^{ns}	577.53^{ns}	114.98*	190.32^{ns}	587.94^{ns}	5.09^{ns}
S × C	4	1117.59^{ns}	1546.54^{ns}	2138.15**	98.70**	132.18^{ns}	1070.16^{ns}	16.78^{ns}
S × P × C	4	27.01^{ns}	66.44^{ns}	22.87^{ns}	5.50^{ns}	3.71^{ns}	6.62^{ns}	0.06^{ns}
Error	36	463.84	1167.94	158.73	19.46	146.58	438.92	1.34
CV (%)		3.18	4.98	1.83	1.83	2.24	4.90	1.44

ns,* and **: non-significant and significant at 5% and 1 % probability levels, respectively; SOD: Superoxide dismutase, POX: Peroxidase, CAT; catalase, MDA: Malondialdehyde.

ZDR planting patterns, which were significantly lower than the weedy check and most of them were not significantly different from the pesticide application (Table 4).

The row spacing × cover crop interaction was significant for the grain yield of maize. Due to favorable growth conditions, the highest grain yield of maize was achieved for the rye cover crop in the 65 cm row spacing (8.04 t ha^{-1}), and it was significantly higher than for the rye cover crop in the 75 cm row spacing (7.35 t ha^{-1}) (Table 3). The lowest grain yield of maize was achieved for the weedy check in the 75 cm row spacing (6.19 t ha^{-1}) (Table 3). On average, the 65 cm row spacing showed about 5.02% higher grain yield than the 75 cm row spacing. A key challenge for farmers using wider maize row spacing is the potential drop in yield per unit area compared with the conventional 65 cm spacing.

Reducing row spacing enhances maize tolerance to high plant density by improving canopy structure and its interactions with environments, such as reducing soil evaporation and optimizing light distribution (Gao *et al*. 2021). In the present study, with decreased row spacing (65 cm), grain yield improved, which is consistent with the results of Porter *et al.* (1997), Johnson and Hoverstad (2002), Widdicombe and Thelen (2002), Shapiro and Wortmann (2006), Williams *et al.* (2014), and Nelson *et al.* (2015).

The rye cover crop resulted in the highest grain yield of maize among the cover crops at both row spacings (Table 3). A cover plant such as rye, due to its rapid establishment and growth capacity in field, is more suitable and effective for weed control. In fact, maize, due to its low growth rate and establishment in the early growing season, cannot compete with weeds (Moore *et al*. 2014). Rye can take up residual or newly mineralized nitrogen, typically accumulating 28-56 kg N ha^{-1} in its roots and shoots (Kaspar *et al*. 2007; Kaspar *et al*. 2012). In addition, rye cover crops have been associated with a 48% reduction in nitrate leaching in drainage water over five years (Kaspar *et al*. 2012). In this study, weed biomass declined at the 65 cm spacing when rye was present (Table 3). The highest grain yield of 8.04 t ha^{-1} was achieved in 65 cm row spacing when mixed with rye. This may be due to the extensive fibrous root system of cereal rye, which reduces erosion (Kaspar *et al*. 2001), improves soil structure, and mitigates soil compaction over time (Kessavalou and Walters 1999). Rye cover crops provide extra benefits, including increased soil organic matter and enhanced weed suppression (Teasdale *et al*. 1991; Moore *et al*. 2014).

The analysis of variance of data indicated significant main effects of row spacing, cover crop, and planting pattern on MDA (Table 2). The recorded MDA values were 35.50 and 32.23 (nmol TBARS g^{-1} FW) at 65 cm and 75 cm, respectively. MDA levels of CSR and ZDR planting patterns were 38.92 and 31.37 (nmol TBARS g^{-1} FW), respectively. The lowest and the highest MDA among cover crops were observed for rye (23.16 nmol TBARS g^{-1} FW) and MaisTer (59.69 nmol TBARS g^{-1} FW), respectively (Table 5). However, for H_2O_2, the interaction of planting pattern × cover crop was also significant (Table 2). The H_2O_2 content was 113.35 nmol g^{-1} FW in the 75 cm row spacing and 134.13 nmol g^{-1} FW in the 65 cm row spacing. The highest and the lowest H_2O_2 content of maize leaves were obtained in MaisTer + CSR (170.85 nmol g^{-1} FW) and weedy check + ZDR (92.85 nmol g^{-1} FW), respectively (Table 6).

The proline content of maize was affected by row spacing, planting pattern, and cover crop (Table 2). The proline content of the 65 cm row spacing was higher compared to the 75 cm row spacing in maize leaves. The proline content was higher in the CSR planting pattern (73.78 µmol g^{-1} FW) compared to the proline content in the ZDR planting pattern (69.49 µmol g^{-1} FW). The highest proline

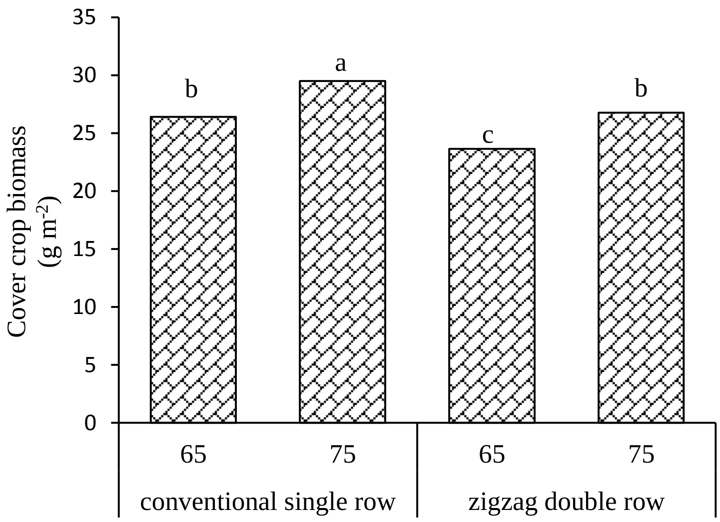

Figure 1. Changes in the cover crop biomass as influenced by various planting patterns and row spacings of maize; Means with different letters are significantly different at $p \leq 0.05$, according to Duncan's multiple range test.

Table 3. Changes in the cover crop biomass, weed biomass, and grain yield of maize as influenced by various cover crops and row spacings.

Row spacing	Cover crop	Cover crop biomass (g m^{-2})	Weed biomass (g m^{-2})	Grain yield (t h^{-1})
65 cm	Weedy check	0f	32.62c	6.29de
	MaisTer	0f	20.86de	7.22bc
	Rye	44.83c	10.40f	8.04a
	Vetch	42.94cd	11.73f	6.66cde
	Clover	37.44e	15.82ef	7.17bc
75 cm	Weedy check	0f	59.62a	6.19e
	MaisTer	0f	40.39b	6.58cde
	Rye	50.96a	27.06cd	7.35b
	Vetch	48.37b	24.48d	6.41de
	Clover	41.33d	25.35d	7.05bcd

Means with different letters in each column are significantly different at $p \leq 0.05$, according to Duncan's multiple range test.

Table 4. Changes in the cover crop biomass and weed biomass as influenced by various planting patterns of maize and cover crops.

Planting patterns	Cover crop	Cover crop biomass (g m^{-2})	Weed biomass (g m-2)
CSR	Weedy check	0f	52.42a
	MaisTer	0f	35.78bc
	Rye	50.48a	26.04cd
	Vetch	47.91b	19.93d
	Clover	41.42d	20.79d
ZDR	Weedy check	0f	39.83b
	MaisTer	0f	25.47cd
	Rye	45.31bc	16.84d
	Vetch	43.40cd	14.95d
	Clover	37.35e	16.29d

Means with different letters in each column are significantly different at $p \leq 0.05$, according to Duncan's multiple range test; CSR: Conventional single row, ZDR: Zigzag double-row.

Table 5. Changes in proline, MDA, and CAT in maize as influenced by various cover crops.

Cover crop	MDA (nmol TBARS g^{-1} FW)	Proline (µmol g^{-1} FW)	CAT (Densitometric activity)
Weedy check	35.49b	74.13a	457.29b
MaisTer	59.69a	74.82a	603.93a
Rye	23.16e	68.37c	305.07e
Vetch	26.59d	70.22b	350.89d
Clover	31.87c	70.62b	421.02c

Means with different letters in each column are significantly different at $p \leq 0.05$, according to Duncan's multiple range test; MDA: Malondialdehyde, CAT: Catalase.

content among cover crops was obtained for MaisTer (74.82 µmol g^{-1} FW), and the weedy check (74.13 µmol g^{-1} FW), and the rye cover crop resulted in significantly lower proline content in the maize leaves than the others (Table 5). The total phenols of maize were affected by row spacing, planting pattern, and type of cover crop. Further, the interaction between the planting patterns and cover crops was significant concerning total phenols (Table 2). Mean of total phenols was 0.924 and 0.359 (mg GA g^{-1} FW) for the 65 cm and 75 cm row spacing, respectively. The average total phenols across planting pattern × cover crop interaction altered from 0.51 mg GA g^{-1} FW (ZDR + weedy check) to 0.87 mg GA g^{-1} FW (CSR + MaisTer) (Table 6). On average over planting patterns, total phenols ranged from 0.69 mg GA g^{-1} FW (CSR planting pattern) to 0.59 mg GA g^{-1} FW (ZDR planting pattern).

The total protein was affected by row spacing, planting pattern, and cover crop. There was also a significant planting pattern × cover crop interaction (Table 2). The total protein in 75 cm row spacing was 0.145 (mg g^{-1} FW), and in 65 cm row spacing was 0.671 (mg g^{-1} FW). With regard to planting pattern × cover crop interaction, the total protein ranged from 0.390 mg g^{-1} FW (CSR + weedy check) to 0.445 mg g^{-1} FW (CSR + rye) (Table 6).

The antioxidant enzymes' assessments revealed the presence of one, two, and three isoforms for CAT, POX, and SOD, respectively (Figure 2). There was a significant interaction between planting patterns and cover crops for SOD1, SOD2, and SOD3 (Table 2). The highest SOD1, SOD2, and SOD3 activities of maize leaves were obtained in MaisTer + CSR as 1183.25, 1371.61, and 1547.50 densitometric activity, respectively (Table 6). In addition, a significant row spacing × cover crop interaction was obtained for the SOD3 activity (Table 2). The SOD3 activity ranged from 1007.85 densitometric activity for the 75 cm row spacing + rye to 1640.18 densitometric activity for the 65

Table 6. Changes in the H_2O_2, total phenols, SOD, POX, and total soluble proteins of maize as exposed to various planting patterns and the cover crops.

Planting pattern	Cover crop	H_2O_2 (nmol g^{-1} FW)	Total phenols (mg GA g^{-1} FW)	Densitometric activity					Total protein (mg g^{-1} FW)
				SOD_1	SOD_2	SOD_3	POX_1	POX_2	
CSR	Weedy check	100.16fg	0.59d	857.50fg	993.97cd	1211.40de	211.78de	243.27d	0.390e
	MaisTer	170.85a	0.87a	1183.25a	1371.61a	1547.50a	285.97a	335.64a	0.428b
	Rye	118.08de	0.64c	1057.68bc	995.71cd	1213.85cde	256.23bc	278.02bc	0.445a
	Vetch	115.11def	0.63c	986.08c	1143.03b	1289.63c	243.44c	279.72bc	0.411c
	Clover	138.170c	0.73b	1117.57ab	1133.93b	1348.68bc	292.51a	352.21a	0.397d
ZDR	Weedy check	92.85g	0.51e	822.20g	871.95d	1075.10e	173.80f	204.23e	0.380f
	MaisTer	153.06b	0.65c	995.55c	1087.63bc	1263.86cd	274.32ab	295.49b	0.415c
	Rye	114.38def	0.60d	973.52cd	954.91cd	1084.07e	233.57cd	255.36cd	0.425b
	Vetch	106.74efg	0.55de	945 50ef	1002.70cd	1236.57cd	199.81ef	234.83de	0.393de
	Clover	128.04cd	0.61cd	1134.70ab	1203.01b	1483.55ab	239.79cd	282.01bc	0.393de

Means with different letters in each column are significantly different at $p \leq 0.05$, according to Duncan's multiple range test; CSR: Conventional single row, ZDR: Zigzag double-row; SOD: Superoxide dismutase, POX: Peroxidase.

cm row spacing + MaisTer (Table 7). The analysis of variance of data showed significant effects of row spacing, cover crop, and planting pattern on POX1 activity. Furthermore, the interaction of row spacing × planting pattern, row spacing × cover crop, and planting pattern × cover crop was significant concerning POX1 (Table 2). MaisTer displayed the highest POX1 activity (303.37 densitometric activity) in the 65 cm row spacing, and the lowest POX1 activity was seen in the 75 cm row spacing with rye (176.73 densitometric activity) and vetch (208.80 densitometric activity) (Table 7). MaisTer and clover displayed the highest POX1 activity in the CSR planting pattern as 285.97 and 292.51 densitometric activity, respectively (Table 6). The lowest and the highest POX1 activity were obtained in the 75 cm row + ZDR (205.68 densitometric activity) and in the 65 cm row spacing + CSR (279.40 densitometric activity), respectively (Figure 3). The effect of row spacing, planting pattern, and cover crop, and the planting pattern × cover crop interaction on the POX2 activity was significant. The POX2 activity ranged from 352.21densitometric activity for CSR planting pattern + clover cover crop to 204.23 densitometric activity for ZDR planting pattern + weedy check (Table 6). The CAT activity of maize was affected by row spacing, planting pattern, and cover crop (Table 2 The CAT activity of maize was higher in the CSR planting pattern (465.77 densitometric activity) compared to the ZDR planting pattern (389.52 densitometric activity). The highest CAT was obtained for the MaisTer (603.93 densitometric activity) among cover crop treatments (Table 5).

MDA, a byproduct of lipid peroxidation, is a reliable indicator of membrane damage (Zheng *et al*. 2022), and the increased electrolyte leakage reflects enhanced membrane permeability. Herbicide treatment, especially at 1.5 L ha^{-1}, elevated the MDA levels significantly (Table 5). Our results were in line with the reports linking higher herbicide concentrations to MDA accumulation (Kırıcı *et al.* 2021). In a study by Yuan *et al*. (2023), H_2O_2 and O_2^- concentrations in maize rose progressively from flowering to maturity. The addition of different herbicides also led to a significant increase in H_2O_2 and O_2^- at maturity.

Proline accumulation is a common response to various stressors (Hasan *et al*. 2022), serving roles in osmotic adjustment, energy storage, and as a carbon and nitrogen source. It also acts as a ROS scavenger, protecting cellular membranes and enzymatic systems (Talaat *et al*. 2015). In this study, proline content increased significantly in MaisTer (Table 5), corroborating findings that herbicides stimulate proline accumulation (Zheng *et al*. 2006). The proline level elevates under various abiotic stresses, including herbicide application (Sergiev *et al*. 2020; Hasan *et al*. 2022).

Herbicide use is an essential component of maize cultivation for weed management (Hanif *et al*. 2021). However, improper or excessive use of herbicides can reduce the levels of SOD, CAT, and

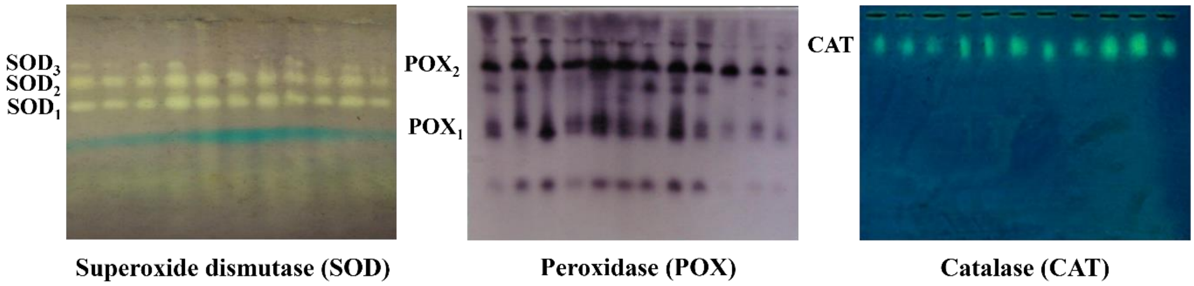

Figure 2. The banding pattern of superoxide dismutase, peroxidase, and catalase isoforms of maize on the polyacrylamide gels in maize.

Table 7. Changes in SOD_3 and POX_1, and total soluble proteins of maize as influenced by various between-row spacing patterns and cover crops.

Row spacing	Cover crop	SOD_3 (Densitometric activity)	POX_1 (Densitometric activity)
65 cm	Weedy check	1390.86b	265.21bc
	MaisTer	1640.18a	303.37a
	Rye	1054.39e	203.20e
	Vetch	1188.65cd	224.56de
	Clover	1243.52c	244.07cd
75 cm	Weedy check	1367.04b	256.97c
	MaisTer	1413.75b	288.23ab
	Rye	1007.85e	176.73e
	Vetch	1159.16d	208.80e
	Clover	1198.79cd	240.05cd

Means with different letters in each column are significantly different at $p \leq 0.05$, according to Duncan's multiple range test; SOD: Superoxide dismutase, POX: Peroxidase.

chlorophyll (Hasan *et al*. 2022). Enhanced activities of SOD, POX, and CAT, along with higher proline content, may be associated with lower MDA levels and improved chlorophyll content (Hanif *et al*. 2021). Additionally, Zheng *et al*. (2022) reported that a narrow row planting pattern elevates the activities of SOD, POX, and CAT in maize leaves during flowering and grain filling, while reducing MDA levels. It has been demonstrated that increasing MDA content leads to lower activities of antioxidant enzymes (SOD, POX, and CAT) in plants cultivated with cover crops (Vesna *et al*. 2016; Dauphinee *et al.* 2017; Brenna and Acosta-Martinez 2019). Our study indicates that using cover crops boosts maize yield by improving weed control and reducing MDA and H_2O_2. These findings align with Yuan *et al*. (2023). However, in our experiment the antioxidants such as SOD, POX, CAT,

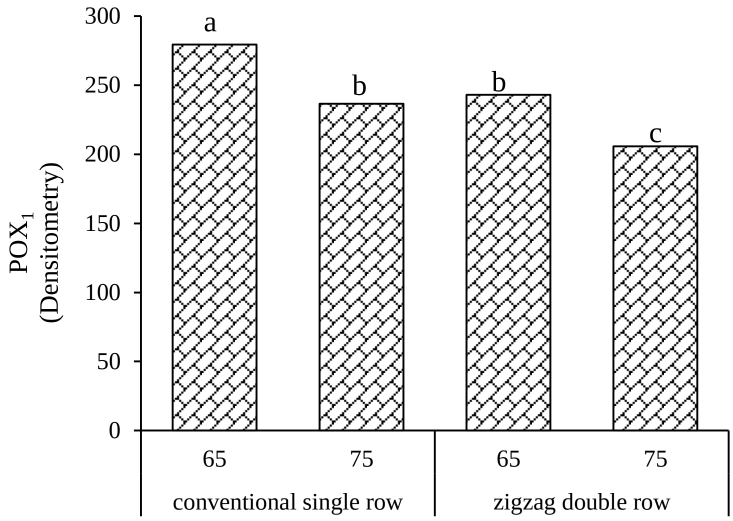

Figure 3. Changes in the peroxidase (POX_1) as influenced by various planting patterns and row spacings of maize; Means with different letters are significantly different at $p \leq 0.05$, according to Duncan's multiple range test.

and proline were also reduced using the rye cover crop as compared to the weedy check and herbicide. May be the rye cover crop reduces the weed stress and, therefore, declines the MDA level in maize, and the plant does not need higher levels of antioxidants for scavenging the ROS.

Narrowing the row spacing and planting configuration may slow the aging process in maize, helping leaves stay green longer and enabling more dry matter to be allocated to the grains, which contributes to higher yields. The use of herbicides also correlates with increased activities of SOD, POX, and CAT in maize leaves. It is widely accepted that having multiple enzymes capable of the same catalytic function enhances a plant's adaptability, a key factor in maintaining essential processes under stress.

Conclusion

Conventional row spacing (75 cm) and CSR planting generally increased the cover crop biomass, and rye performed best under favorable spacing/planting combinations. Weed biomass and grain yield were affected by row spacing and cover crop interactions, with the 65 cm row spacing supported higher grain yield when rye was used. The data analysis reveals that row spacing, cover crop, and planting pattern significantly affected MDA, H_2O_2, proline, total phenols, and total proteins in maize. Proline, total protein, total phenols, MDA, and H2O2 were greater at the 65 cm row spacing. On the other hand, the rye cover crop decreased the MDA and H_2O_2 content as compared to the weedy check and herbicide.

Author Contributions

All authors made equal contributions to the conceptualization, writing, editing, and review of the manuscript. Furthermore, all authors have provided their consent for the submission of the current manuscript.

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Conflict of Interest

The authors declare that they have no conflict of interest.

Data Availability Statements

All available data are inside the article.

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