



Integrated application of nitrogen and mepiquat chloride improves yield of *Brassica napus* L. by modulating shattering, lodging and nitrogen use efficiency under rainfed conditions

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Abstract

Nitrogen is the major yield-limiting factor for *Brassica napus* but is often deficient in soils. Excessive and inefficient nitrogen application can lead to lodging, reduced nitrogen use efficiency, and increased shattering losses during mechanical harvesting. However, co-application of mepiquat chloride and nitrogen can be a viable agronomic intervention to alleviate these challenges. A two-year field study (2022–23 and 2023–24) with factorial randomized complete block design (RCBD) was conducted to unravel effects of nitrogen (0, 85, 170, 255 kg ha⁻¹) conjugated with foliar application of mepiquat chloride (0, 50, 100 mg L⁻¹) on shattering, lodging and nitrogen use efficiency (NUE) of *B. napus*. Increasing nitrogen from 0 to 255 kg ha⁻¹ significantly increased plant height, biological yield, lodging and shattering; however, integration of nitrogen with mepiquat chloride (MC) abridged these traits. In both years (2022–23 and 2023–24), 170 kg ha⁻¹ nitrogen combined with 50 mg L⁻¹ MC resulted in significantly higher number of pods plant⁻¹ (375.88, 356.13), seeds pod⁻¹ (24.30, 21.83), 1000-seed weight (3.84 g, 3.65 g), chlorophyll content (8.84 mg g⁻¹, 8.49 mg g⁻¹), seed yield (2362 kg ha⁻¹, 2260 kg ha⁻¹), NUE matrixes viz.; apparent recovery (53.68%, 51.57%), agronomic efficiency (6.36 kg kg⁻¹, 5.73 kg kg⁻¹), utilization efficiency (13.64 kg kg⁻¹, 13.45 kg kg⁻¹), agro-physiological efficiency (11.83 kg kg⁻¹, 11.14 kg kg⁻¹), and antioxidant activity, but lower lipid peroxidation (MAD). Lodging was positively correlated with plant height ($r=0.85$, 0.71), biological yield ($r=0.94$, 0.89) and nitrogen uptake efficiency ($r=0.75$, 0.75), while seed yield was negatively correlated with shattering ($r=-0.74$, -0.64) and malondialdehyde (MDA) content ($r=-0.72$, -0.86). This is the first report demonstrating that combined nitrogen and MC application reduces shattering and lodging in *B. napus*, while improving yield. Applying 170 kg ha⁻¹ nitrogen and 50 mg L⁻¹ MC at rosette and silique formation stages is recommended for higher seed yield in water-deficient environments.

Keywords Antioxidant · *Brassica napus* · Pod shattering · Lodging · Plant growth regulators · Nitrogen use efficiency

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Introduction

Canola (*Brassica napus* L.) is an important oilseed crop of the world including Pakistan (Noreen et al. 2016). Pakistan meets only 17% of its edible oil needs through local production of oilseed crops, while the remaining demand of 83% is satisfied through import. The demand and import for high-quality oil are steadily rising with increasing population. The gap between demand and supply of edible oil has forced the country to import 2.71 million tonnes of edible oil worth US \$ 2.809 billion. Increasing reliance on edible oil import underscores the importance of enhancing domestic production of oilseed crops in the country. Increasing inland cultivation of Canola may contribute to the diversification of the country's agricultural landscape, along with self-sufficiency of edible oil and curtailing the import bill.

Canola seed contains 40% good quality oil (Ahmad et al. 2020) with low level of erucic acid (<2%) and glucosinolates (<30 μmol per gram of rapeseed meal), moderate amount of polyunsaturated fatty acids, substantial content of tocopherols, phytosterols, and omega-3 fatty acids, and high contents of monounsaturated fatty acids. Canola oil reduces the chances of type II diabetes, heart diseases, hypercholesterolemia, and is considered one of the most nutritionally balanced edible vegetable oils by today standards (Goyal et al. 2021).

Nitrogen is the largest and indispensable input for Canola production (Gan et al. 2008). Almost 100% soils of Pakistan are deficient in nitrogen and reliance on nitrogenous fertilizers is unavoidable. Approximately 30–35% of the nitrogen used for crop production is lost to the environment (Swify et al. 2023). The higher doses of nitrogenous fertilizers may even result in 50–70% N losses through NH_3 volatilization and NO_3^- leaching (Aziz et al. 2019) and reduces effectiveness of applied nitrogen. Therefore, nitrogen management strategies which can increase nitrogen use efficiency (NUE) along with safeguarding the environment are needed to ensure maximum seed yield and seed quality of canola (Ma and Herath 2016).

Nitrogen is a building block of amino acids, membrane proteins, chlorophyll, enzymes, coenzymes, plant growth regulators, amides, nucleic acid, auxins, cytokinin (Ganie et al. 2017). Also, it is an integral constitute of antioxidants including superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD) which scavenge reactive oxygen species and enable the plants to cope with abiotic stress. Nitrogen application therefore plays a key role in plant growth, development, oil yield and to harvest potential seed yield of *B. napus*. An improvement in seed yield by 50.33% is reported with N application of 90 kg N ha⁻¹ with respect to zero N fertilization in *Brassica napus* (Yahbi et al. 2022). However, both suboptimal and excessive nitrogen supply

significantly reduces the yield of *Brassica napus* (Yahbi et al. 2022; Berry et al. 2010). Excessive nitrogen supply results in more vegetative growth, over-heighted plants, less yield and increases likelihood of shattering and lodging in *Brassica napus*. Lodging and shattering hinder the mechanical harvesting of canola cultivated on large scale making mechanical harvesting impracticable and resilience on manual harvesting is unavoidable. Over-ripening of silique due to delay in harvesting operations may lead to pod shattering (Sintim et al. 2016).

Timely harvesting of canola grown on a large scale is crucial for *Brassica napus* production, and this is only feasible by machine harvesting. However, the excessive plant height, prolonged and uneven seed ripening, lodging and shattering make mechanical harvesting of crop challenging (Hobson and Bruce 2002). Also, the over-heighted plants and a large number of primary branches due to higher nitrogen inputs typically result in a lack of uniform maturation between the main inflorescences and primary branches which leads to the delay in harvesting. The above challenges and opportunities indicated that nitrogen and an ideal crop architecture is needed for mechanical harvesting, reduced incidence of shattering with resultant maximum yield of *Brassica napus*.

Mepiquat chloride (MC), an anti-auxin and gibberellin plant growth regulator, could be effectively used to reduce plant height, the risk of lodging (Polat et al. 2017) and yield improvement by modulating plant architecture (Cook and Kennedy 2000). It retards cell division, arrests hyper elongation of cell, internode length, growth, apical dominance and prevents plants from becoming too tall or spindly by regulating auxin and gibberellic acid concentration (Wang et al. 2014; Tung et al. 2020). MC improves structural and stability of plants and tends to reduce likelihood of lodging. Therefore, it is commonly applied with nitrogen in various crops to halt the vegetative growth, induce flowering, mitigate lodging and maximizing yield (Biles and Cothren 2001; Tung et al. 2020; Abbas et al. 2022).

Little information is available regarding the effect of conjugate application of soil applied N with foliar application of Mepiquat chloride (MC) on yield and shattering tendency and nitrogen use efficiency (NUE) of *B. napus*. It was hypothesized that the precise combination of nitrogen and MC will reduce shattering, improve NUE and yield of *Brassica napus*. Considering the challenges and opportunities highlighted, present study was conducted to explore potential of combined application of MC and nitrogen on shattering, NUE and yield of *Brassica napus* L. under rain-fed conditions.

Materials and methods

Experimental site

The field experiments were conducted at PMAS Arid Agriculture University Research Farm, Chakwal Road, Punjab, Pakistan (33.117229 N, 73.011748 E) from October–March 2022–2023 and 2023–2024. The climate of the experimental site was characterized with high temperatures and frequent drought spells at various growth stages of crop. The crop was raised under rainfed conditions and no supplemental irrigation was applied at any growth stage of crop. During the 2022–23 and 2023–24 season, the crop received 95.39 mm and 97.29 mm rainfall respectively, distributed throughout crop phenological progression. In 2023–24, more rainfall was received in October which provided ideal moisture for germination and initial stand establishment of crop than 2022–23. The weather data (rain, temperature) during the growing seasons 2022–23 and 2023–24 are provided in Fig. 1.

Experimental design and field operations

The experiments were laid out in randomized complete block design (RCBD) with factorial arrangement and replicated four times. The canola variety “Chakwal Sarsoon” generally susceptible to lodging was used as a plant material using seed rate of 6.0 kg ha⁻¹. Prior to the onset of monsoon season (July–September), the field was ploughed using a moldboard plow and then left fallow. At the end of the rainy season, the field was planked to conserve soil moisture. The crop was sown on conserved soil moisture on 10th October and 8th October of 2022–23 and 2023–24 respectively, with a single-row hand-operated drill at seeding depth of 3 cm, keeping row to row distance of 45 cm. After seedling establishment, plant-to-plant distance of 25 cm was maintained. Basal doses of phosphorous and potassium were applied at the 60 and 30 kg ha⁻¹ respectively at the time of seedbed preparation. No herbicides or pesticides were applied during the experimental period; instead, weeds were controlled by regular hand weeding. The treatments consisted of 0, 50, 100 mg L⁻¹ of mepiquat chloride and 0, 85, 170, 255 kg ha⁻¹ of nitrogen. The entire N as per treatments was applied once as a basal application, while mepiquat chloride (MC) was foliar applied at the rosette and repeated at silique formation stage, as per treatments. Plain water was sprayed in control treatment (MC₀). Pre-sowing physico-chemical properties of the soil are presented in Table 1.

Plant sampling for physiological and biochemical analysis

After two weeks of the second application of Mepiquat chloride (MC), the leaf samples (upper fully expanded leaves) were collected early in the morning, immediately packed in zip-lock bags, and carried to the laboratory in cooler with ice packs. For biochemical analyses, samples were frozen in liquid nitrogen until further processing.

Physiological traits of plants

Relative water content (RWC %)

The RWC was computed as proposed by (Barrs and Weatherley 1962). Two plants from each treatment combination for each replication were selected and RWC of upper fully expanded leaves were recorded and averaged. The fresh weight (FW) of leaf discs was recorded immediately after sampling. The samples were then placed in distilled water for 4 h to record the turgid weight (Tw). Subsequently, samples were placed in a hot air oven until constant weight and dry weight (Dw) was recorded. The RWC (%) was calculated by formula given below:

$$RWC (\%) = \frac{(FW - DW)}{(TW - DW)} \times 100$$

Chlorophyll content (mg g⁻¹)

Two plants from each treatment combination for each replication were selected for estimation of average chlorophyll contents. The chlorophyll “a” and “b” were determined following method of (Arnon 1949). For this purpose, 0.1 g of leaf sample were collected and homogenized in 10 ml of 80% acetone and kept in the dark overnight. After 24 h, sample solution was centrifuged at 10,000 rpm for 30 min. The absorbance of the supernatants was measured at 663 and 645 nm wavelength in a spectrophotometer. The total chlorophyll contents were calculated by the formula given below:

$$\begin{aligned} &\text{Chlorophyll content (mg g}^{-1}\text{)} \\ &= 20.2(A_{645}) - 8.02(A_{663}) \times \frac{V}{1000 \times W} \end{aligned}$$

Where; A absorbance at specific wavelengths, while V represents the final volume of chlorophyll extract and W is the fresh weight of tissue extract.

Fig. 1 Weather data of experimental site for crop during growing season 2022-23 and 2023-24

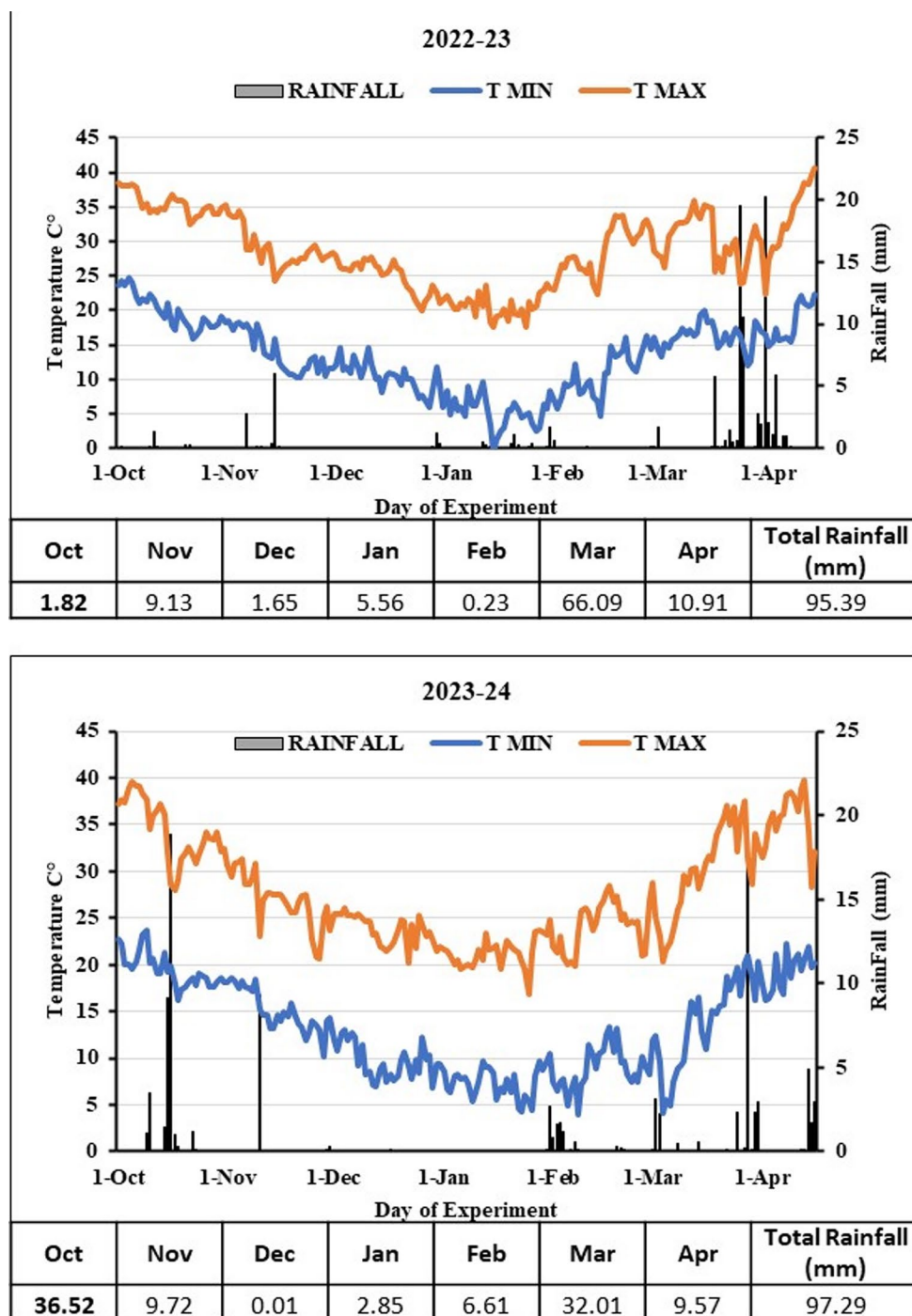


Table 1 Physico-chemical properties of soil before sowing

Characteristics	Year 2022-23	Year 2023-24
Texture	Loam	Loam
EC _{1:2} (dSm ⁻¹)	0.80	0.87
pH	7.58	7.45
Organic matter (%)	1.33	1.17
NO ₃ -N (mg kg ⁻¹)	3.34	3.81
Olsen P (mg kg ⁻¹)	4.2	3.67
Available K (mg kg ⁻¹)	103	66

Antioxidant enzymatic activities

The fresh leaf tissues (0.5 g) were used for quantification of catalase (CAT) and Superoxide dismutase (SOD) activity. Catalase (CAT) activity was quantified spectrophotometrically at 240 nm by using the method of (Aebi 1984). One unit of the catalase activity was defined as the activity required to consume 1 $\mu\text{mol H}_2\text{O}_2$ in 50 mM phosphate buffer (pH 7.0) at 25 °C in 30 (Tepe and Aydemir 2011) and

was expressed as unit mg^{-1} protein. Superoxide dismutase (SOD) activity was assayed and quantified by monitoring superoxide radical-induced nitroblue tetrazolium reduction at 560 nm. One unit of SOD activity was defined as the amount of enzyme, causing 50% inhibition of this reaction in comparison with a blank sample and expressed as units mg^{-1} protein (Beauchamp and Fridovich 1971; Kong et al. 2017; Tepe and Aydemir 2011).

Lipid peroxidation/malondialdehyde ($\mu\text{mol g}^{-1}$ FW)

The extent of lipid peroxidation was determined based on malondialdehyde contents ($\mu\text{mol g}^{-1}$ FW). The malondialdehyde (MDA) was quantified following reaction with Thiobarbituric acid (TBA) (Tepe and Aydemir 2011; Heath and Packer 1968). For this assay, fresh plant samples (0.5 g) were homogenized in 1% trichloroacetic acid and centrifuged at 10,000 rpm for 15 min. The supernatant was heated with 0.05 Thiobarbituric acid for 30 min at 95 °C, followed by re-centrifugation at 5000 rpm for 10 min. The absorbance was recorded at 532 and 600 nm. MDA was determined with an extinction coefficient of $155 \text{ mM}^{-1} \text{ cm}^{-1}$ given for 1 g fresh weight as per equation below.

$$\text{MDA} = \frac{A_{532} - A_{600}}{155}$$

Morphological and yield traits

At physiological maturity (April 01, 2022–23 and April 19, 2023–24), ten plants from each treatment plot were randomly selected and morphological traits including plant height, primary and secondary branches were counted and average of counts was taken as the final value. At harvest time (April 11, 2022–23 and April 02, 2023–24), the number of pods were counted on each plant and fifty pods from each treatment were taken, threshed manually to count the seed numbers in each selected pot and average value was taken. For determining the grain weight, 1000 seeds were collected from each treatment and average weight of three replicates was noted as the final value. The experimental plants were mechanically harvested and sun dried by keeping them upright. After sun drying, plants were threshed for calculating seed, while the samples were oven dried for determining straw biomass yield (kg ha^{-1}).

Lodging and shattering (%)

Plants were considered as lodged when the angle of their stem exceeded 45° relative to the vertical position. Lodging was expressed as a percentage of plants lodged to the total number of plants in each plot. At physiological maturity,

Pods of ten randomly tagged plants were counted, and the number of intact and unopen pods were recorded. The plots were mechanically harvested and shattering percentage was calculated by formula below:

$$\begin{aligned} \text{Shattering (\%)} \\ &= \frac{\text{Number of intact and unopened pods after harvesting}}{\text{Total number of intact pods before harvesting}} \times 100 \end{aligned}$$

Nitrogen use efficiency matrices

The plant samples were collected after harvesting, the oven dried, grinded and digested for determination of plant nitrogen content by Kjeldahl method. The nitrogen apparent recovery (NAR), agronomic efficiency (AgE), agro-physiological efficiency (AgPE), nitrogen utilization efficiency (UTE) and partial factor productivity (PFP) were calculated as explained by Hasanali deh and Hojati (2012) and Mahmood et al. (2024).

Nitrogen apparent recovery efficiency (%)

The nitrogen apparent recovery efficiency (NAR) represents the percentage of nitrogen taken up by the plant (kg) from that applied in the soil and was determined according to the following equation.

$$\begin{aligned} \text{NAR (\%)} \\ &= \frac{\text{N uptake in fertilized plot} - \text{N uptake in control plot}}{\text{N applied}} \times 100 \end{aligned}$$

Agronomic efficiency (kg kg^{-1})

Agronomic nitrogen use efficiency (AgE) represents grain yield obtained per unit of nitrogen applied and was calculated as per equation below.

$$\text{AgE} = \frac{\text{Yield in fertilized Plot} - \text{Yield in control plot}}{\text{Nitrogen applied}}$$

Nitrogen utilization efficiency (kg kg^{-1})

Nitrogen utilization efficiency (UTE) refers to the ability of plants to produce economic yield (grain) in response to total nitrogen uptake (applied + soil mineral nitrogen) by plants. The UTE was computed by the following equation.

$$\text{UTE} = \frac{\text{Grain Yield}}{\text{Nitrogen uptake}}$$

Agro-physiological efficiency (kg grain kg⁻¹ N uptake)

Agro-physiological efficiency of nitrogen (AgPE) is the ratio of grain yield per unit nitrogen uptake in the above ground biomass at maturity. The AgPE was calculated as per following equation:

$$\text{AgPE (kg/kg)} = \frac{\text{Grain yield with } N_t - \text{Grain yield with } N_0}{N \text{ uptake in } N_t - N \text{ uptake in } N_0}$$

Where, N_t is nitrogen application rate, N_0 is control (no nitrogen).

Partial factor productivity (kg kg⁻¹)

The partial factor productivity (PFP) is the ratio of crop yield per unit of nitrogen applied and was computed by using the following equation:

$$\text{PFP} = \frac{\text{Grain Yield}}{\text{Nitrogen applied}}$$

Data analysis

The data were analyzed using Fisher's Analysis of Variance technique, using the statistical software Statistix Version 8.1. The differences among means were compared by Least Significant Difference (LSD) at 5% probability (Steel and Torrie 1981).

Results

Plant height (cm)

During both years of the study (2022–23 and 2023–24), the interaction between nitrogen (N) and mepiquat chloride (MC) showed non-significant effect on plant height ($P > 0.05$), as shown in Table 2. In 2022–23, increasing nitrogen from N_0 to N_{255} resulted in increased plant height from 132.38 cm to 176.82 cm (33.57% increase over N_0). Conversely, increasing mepiquat concentration from MC_0 to MC_{100} significantly reduced plant height from 166.92 cm to 144.00 cm—a reduction of 13.73%. A comparable response was observed in 2023–24, following a similar trend where tallest plants were observed in response to N_{255} (172.37 cm), while the shortest were experienced with MC_{100} (143.56 cm).

Number of branches plant⁻¹

The data (Table 2) revealed that interaction between N and MC on number of primary and secondary branches was non-significant during both years ($P > 0.05$). However, the main individual effect of nitrogen and MC on primary and secondary branches was significant. During the planting year of 2022–23 and 2023–24, the minimum number of primary (6.61 and 6.10) and secondary branches (4.17, 3.91) were recorded in control plots (N_0). Application of N_{255} significantly enhanced plant branching compared to the control (N_0), with the number of primary branches increasing to 11.03 and 10.27 (66.87% and 68.36% higher than N_0), and secondary branches increasing to 6.18 and 5.91 (48.20% and 51.15% higher than N_0) during the 2022–23 and 2023–24 growing seasons, respectively. The increasing concentration of mepiquat chloride from MC_0 to MC_{100} significantly reduced the number of primary branches from 10.29 to 8.38 (18.56% decrease).

and from 9.63 to 7.37 (23.47% decrease) during first and second year of planting, respectively. A similar trend was also witnessed for number of secondary branches during first and second season of study (Table 2), with maximum number of secondary branches in response to MC_0 (6.08, 5.85) and minimum for MC_{100} (4.37, 4.17).

Number of pods plant⁻¹

As indicated by the data presented in Table 2, the interaction of nitrogen and mepiquat chloride (MC) significantly affected number of pods per plant during both years of the study ($P < 0.05$). In 2022–23, the highest number of pods was recorded with the combined application of N_{170} and MC_{100} (393.52), followed by $N_{170} + MC_{50}$ (375.88). In contrast, the individual application of the highest MC dose without nitrogen ($N_0 + MC_{100}$) resulted in the lowest number of pods (183.04), which were about 53.49% lower than that observed under $N_{170} + MC_{100}$. A similar trend was observed in 2023–24, with $N_{170} + MC_{100}$ (369.52) and $N_{170} + MC_{50}$ (356.13) producing the maximum pod numbers, with non-significant difference between them. The lowest pod count (180.68) was again recorded in $N_0 + MC_{100}$ -treated plants, representing a 51.11% reduction compared to $N_{170} + MC_{100}$.

Number of seeds pod⁻¹

The combined application of N and MC significantly influenced the number of seed in each pod ($P < 0.05$) during both years (Table 3). During the 2022–23 season, the highest number of seeds per pod were recorded for $N_{170} + MC_{50}$ (24.30), followed by $N_{170} + MC_{100}$ (23.51). In contrast, the lowest seeds per pod were observed under $N_0 + MC_{100}$ (15.58) and

Table 2 Effect of nitrogen and mepiquat chloride on plant height, primary & secondary branches, and number of pods of pods of *Brassica napus* L.

Treatments	Plant height (cm)		Primary branches plant ⁻¹		Secondary branches plant ⁻¹		Number pod plant ⁻¹	
	2022-23	2023-24	2022-23	2023-24	2022-23	2023-24	2022-23	2023-24
Nitrogen (N)								
N ₀	132.41d	129.82d	6.61c	6.10c	4.17b	3.91b	213.92c	208.02c
N ₈₅	151.81c	148.49c	8.34b	7.87b	4.68b	4.45b	342.91b	291.24b
N ₁₇₀	164.94b	160.55b	10.56a	9.92a	5.57a	5.39a	371.52a	337.29a
N ₂₅₅	176.87a	172.37a	11.03a	10.27a	6.18a	5.91a	335.95b	299.75b
LSD value	5.82*	6.67*	0.79*	0.61*	0.70*	0.54*	11.25*	10.10*
Mepiquat chloride (MC)								
MC ₀	166.92a	161.93a	10.29a	9.63a	6.08a	5.85a	308.50b	263.34c
MC ₅₀	158.11b	152.94b	8.73b	8.62b	4.99b	4.72b	322.58a	300.97a
MC ₁₀₀	144.48c	143.56c	8.38b	7.37c	4.37c	4.17c	317.14ab	287.91b
LSD value	5.04*	5.78*	0.68*	0.53*	0.60*	0.46*	9.74*	8.75*
N × MC (interaction)								
N ₀	142.05±4.36	136.61±8.04	7.62±0.63	6.67±0.29	5.12±0.36	4.92±0.41	250.99±6.56 g	239.92±5.60d
MC ₀	135.41±4.56	131.52±9.01	6.32±0.38	6.06±0.13	4.19±0.57	3.82±0.27	207.75±11.09 h	203.47±10.20e
MC ₅₀	119.76±4.65	121.34±9.80	5.9±0.52	5.6±0.16	3.2±0.09	3.03±0.08	183.04±13.30i	180.68±7.59f
MC ₁₀₀	161.08±7.49	157.92±5.80	9.48±0.25	9.59±0.55	5.83±0.65	5.57±0.22	325.34±11.15ef	276.15±11.83c
N ₈₅	153.36±7.29	148.66±10.04	8.03±0.32	7.4±0.57	4.39±0.51	4.14±0.44	362.77±6.75bc	327.66±6.71b
MC ₀	140.98±5.73	138.9±10.89	7.53±0.67	6.63±0.53	3.83±0.51	3.64±0.4	340.61±7.22de	269.89±4.10c
MC ₅₀	176.69±4.52	170.49±10.57	11.87±0.63	10.82±0.79	6.42±0.78	6.29±0.3	345.15±9.43 cd	286.20±2.88c
MC ₁₀₀	166.44±4.38	159.74±9.12	9.89±0.62	10.47±0.33	5.56±0.56	5.26±0.39	375.88±6.46ab	356.13±4.69a
N ₁₇₀	151.67±4.80	151.43±10.21	9.94±0.31	8.5±0.13	4.75±0.49	4.63±0.37	393.52±6.58a	369.52±6.08a
N ₂₅₅	187.85±6.71	182.70±7.35	12.24±0.42	11.47±0.69	6.97±0.69	6.66±0.34	312.51±4.25f	251.09±4.67d
MC ₀	177.25±4.97	171.86±8.93	10.69±0.28	10.59±0.32	5.85±0.6	5.67±0.26	343.95±9.27cde	316.60±11.13b
MC ₅₀	165.5±2.43	162.55±9.15	10.19±0.41	8.78±0.23	5.74±0.28	5.41±0.22	351.4±6.42 cd	331.55±10.16b
MC ₁₀₀	10.09^{NS}	11.56^{NS}	1.37^{NS}	1.06^{NS}	1.21^{NS}	0.93^{NS}	19.49*	17.50*
LSD(N × MC)								

*Significant, ^{NS} non-significant. Data represent average of four replications± standard error. Different lowercase letters show significant differences ($P \leq 0.05$) among different treatments

$N_0 + MC_{50}$ (16.66), representing decreases of 33.88% and 31.44%, respectively, compared to $N_{170} + MC_{50}$. A similar trend was witnessed during the second year of the study (2023–24), with significantly higher number of seeds in response to $N_{170} + MC_{50}$ (21.83) followed by $N_{170} + MC_{100}$ (21.08). In contrast, the lowest seed number was noted for $N_0 + MC_{100}$ (13.52), which was 38.07% lower than that recorded under $N_{170} + MC_{50}$.

1000-seed weight (g)

The interaction of N and MC on 1000-seed weight was significant during both years ($P < 0.05$) as is evident from the data in Table 3. Treatment combinations either lacking nitrogen (N_0) or receiving excessive nitrogen (N_{255}), in the absence of MC, exhibited comparatively lower 1000-seed weights in both growing seasons. During the 2022–23 season, the highest 1000-seed weights, with no significant difference, were recorded under $N_{170} + MC_{100}$ (3.94 g), followed by $N_{170} + MC_{50}$ (3.84 g) and $N_{85} + MC_{100}$ (3.80 g). A similar trend was observed in 2023–24, with seed test weights of 3.65 g, 3.61 g, and 3.53 g for $N_{170} + MC_{100}$, $N_{170} + MC_{50}$, and $N_{85} + MC_{100}$, respectively (Table 3).

Biological yield (kg ha⁻¹)

The interaction of nitrogen and MC on straw yield was non-significant ($P > 0.05$) across both years (Table 3). Increasing the MC concentration significantly decreased the straw biomass in both years, whereas an increase in nitrogen application led to the opposite effect. The highest straw biomass was obtained from N_{255} treatment, with yields of 6691.70 kg ha⁻¹ in 2022–23 and 6038.50 kg ha⁻¹ in 2023–24. In contrast, the lowest biomass was recorded in the control (N_0) with yield of 4517.90 kg ha⁻¹ and 4280.80 kg ha⁻¹, reflecting reductions of 32.49% and 29.11%, respectively, compared to N_{255} . In contrast, increasing the concentration of mepiquat chloride (MC) from MC_0 to MC_{100} led to a reduction in straw biomass, decreasing from 6005.40 kg ha⁻¹ to 5088.50 kg ha⁻¹ (15.27% decrease) in 2022–23, and from 5672.10 kg ha⁻¹ to 4602.00 kg ha⁻¹ (18.87% decrease) in 2023–24 (Table 3).

Seed yield (kg ha⁻¹)

The interaction between nitrogen and mepiquat chloride (MC) significantly affected seed yield in both growing seasons ($P < 0.05$; Table 3). In 2022–23, the lowest seed yield was observed in response to $N_0 + MC_{100}$ treatment (1166.28 kg ha⁻¹), whereas the highest yield was obtained with $N_{170} + MC_{50}$ (2362.00 kg ha⁻¹), representing a 102.52% increase over $N_0 + MC_{100}$. A similar trend was noted in

2023–24, with $N_{170} + MC_{50}$ again producing the highest yield (2260.33 kg ha⁻¹), which was 96.68% greater than the yield recorded under $N_0 + MC_{100}$ (1149.27 kg ha⁻¹).

Chlorophyll contents (mg g⁻¹)

In both years of study, the combined application of nitrogen and mepiquat chloride ($N \times MC$) demonstrated higher chlorophyll content ($P < 0.05$) than their individual use. Application of $N_{170} + MC_{50}$ resulted in maximum chlorophyll contents of 8.84 mg g⁻¹ and 8.49 mg g⁻¹ during 2022–23 and 2023–24 respectively. The lowest chlorophyll contents of 2.18 mg g⁻¹ and 1.91 mg g⁻¹ were observed during 1st and 2nd year of planting respectively in $N_0 + MC_0$ treated plants, with respective decrease of 75.34% and 77.50% with respect to $N_{170} + MC_{50}$ (Table 3).

Relative water content (%)

The interaction of N and MC on relative water contents (RWC) was non-significant ($P > 0.05$) during both years, however, the main individual effect of nitrogen and MC was significant ($P < 0.05$). In both years, the increasing nitrogen rate from 0 to 170 kg ha⁻¹ resulted in an improvement of RWC, however, further increase in nitrogen (255 kg/ha) reduced RWC (Fig. 2). The maximum RWC of 84.55% and 79.97% was noted in 2022–23 and 2023–24 respectively in response to N_{170} . Similarly, application of mepiquat chloride (100ppm) significantly improved RWC compared with control in both years (Fig. 2).

Superoxide dismutase (unit mg⁻¹ protein)

The interaction of nitrogen and mepiquat chloride significantly affected SOD activity ($p < 0.05$) in both years. In year 2022–23, the maximum SOD activity of 65.74 U mg⁻¹ protein was observed in response to $N_0 + MC_{50}$ followed by $N_0 + MC_{100}$ (64.65 U mg⁻¹ protein) and $N_{255} + MC_{100}$ (65.28 U mg⁻¹ protein) (Fig. 3A, B). Treatments comprising $N_{170} + MC_0$ and $N_{170} + MC_{50}$ resulted in the lowest SOD activity of 49.22 U mg⁻¹ protein and 50.43 U mg⁻¹ protein. In year 2023–24, the maximum SOD activity was verified for plots which received only mepiquat chloride at 50 mg/l or 100 mg/l missing nitrogen supply (Fig. 3A, B).

Catalase (unit mg⁻¹ protein)

The interactive effect of nitrogen and MC was significant during both years of study. The treatments comprising only MC significantly improved CAT activity during both years ($p < 0.05$). The highest CAT activity of 6.47 U mg⁻¹ protein and 6.59 U mg⁻¹ protein was recorded in 1st and 2nd

Table 3 Effect of nitrogen and mepiquat chloride on number of seed pod⁻¹, 1000-seed weight, biological yield, seed yield and chlorophyll contents of Brassica napus L.

Treatments	Number of seed pod ⁻¹		1000 seed weight (g)		Straw biomass (kg ha ⁻¹)		Seed yield (kg ha ⁻¹)		Chlorophyll content (mg g ⁻¹)	
	2022-23	2023-24	2022-23	2023-24	2022-23	2023-24	2022-23	2023-24	2022-23	2023-24
Nitrogen (kg ha⁻¹)										
N ₀	16.61d	14.31c	2.79d	2.55d	4517.90d	4280.80d	1267.30d	1279.80c	2.88d	2.56d
N ₈₅	20.44c	18.44b	3.60b	3.35a	5044.80c	4800.50c	1663.60c	1634.60b	5.87b	5.24b
N ₁₇₀	22.92a	20.62a	3.74a	3.53a	6061.90b	5521.60b	2088.80a	2026.50a	8.36a	7.95a
N ₂₅₅	21.65b	18.92b	3.43c	2.70c	6691.70a	6038.50a	1785.00b	1685.20b	4.91c	4.66c
LSD value	0.79*	0.96*	0.13*	0.14*	160.43*	188.95*	68.05*	62.80*	0.27*	0.26*
Mepiquat chloride (mg L⁻¹)										
MC ₀	19.29b	16.96c	3.25b	3.43a	6005.40a	5672.10a	1578.00c	1564.60c	5.14b	4.71b
MC ₅₀	21.00a	19.16a	3.43a	3.64a	5643.30b	5206.90b	1794.30a	1735.60a	5.80a	5.41a
MC ₁₀₀	20.91a	18.10b	3.49a	3.55a	5088.50c	4602.00c	1731.20b	1669.50b	5.57a	5.19a
LSD value	0.68*	0.83*	0.11*	0.12*	138.93*	163.64*	56.93*	54.39*	0.24*	0.23*
Nitrogen × Mepiquat chloride (Interaction)										
N ₀ MC ₀	17.6±0.59gh	14.17±0.51hi	2.94±0.13f	2.66±0.1ef	4871.41±77.70	4660.32±97.26	1355.88±30.07f	1404.28±57.61f	2.18±0.09g	1.91±0.13g
MC ₅₀	16.66±0.43hi	15.26±0.39gh	2.79±0.11fg	2.56±0.1ef	4564.27±68.69	4284.21±124.33	1279.83±27.94fg	1285.76±34.76g	3.44±0.16f	3.07±0.1f
MC ₁₀₀	15.58±0.25i	13.52±0.55i	2.65±0.11g	2.45±0.1f	4117.89±90.47	3897.97±103.05	1166.28±40.96g	1149.27±30.73h	2.99±0.12f	2.69±0.14f
N ₈₅ MC ₀	18.73±0.26fg	18.28±0.91de	3.31±0.1e	3.19±0.05cd	5479.18±95.45	5403.27±168.68	1604.75±40.88e	1640.03±46.07de	6.04±0.21c	5.16±0.14cd
MC ₅₀	20.86±0.72de	20.04±0.66bc	3.7±0.04bc	3.35±0.03bc	5116.94±55.98	4817.26±92.80	1764.70±18.64d	1712.66±20.55d	5.64±0.29cd	5.09±0.24cd
MC ₁₀₀	21.74±0.22cd	17.02±0.31ef	3.8±0.04abc	3.53±0.05ab	4538.36±142.68	4180.87±106.01	1621.32±37.14e	1551.20±32.33e	5.92±0.29c	5.44±0.16c
N ₁₇₀ MC ₀	20.96±0.33de	18.95±0.41cd	3.47±0.07de	3.36±0.06bc	6535.16±119.88	5998.39±141.05	1713.94±41.89de	1669.70±47.32d	8.26±0.15b	7.81±0.2b
MC ₅₀	24.3±0.55a	21.83±0.29a	3.84±0.05ab	3.65±0.06a	6178.02±229.04	5587.11±142.12	2362.00±61.48a	2260.33±38.39a	8.84±0.17a	8.49±0.18a
MC ₁₀₀	23.51±0.66ab	21.08±0.63ab	3.94±0.03a	3.61±0.1a	5472.56±146.14	4979.42±109.24	2190.45±71.46b	2149.58±29.89b	7.99±0.15b	7.53±0.24b
N ₂₅₅ MC ₀	19.92±0.21ef	16.47±0.76fg	3.29±0.06c	2.42±0.12f	7136.03±255.89	6626.57±295.90	1637.53±36.60e	1544.22±32.29e	4.07±0.16e	3.94±0.26e
MC ₅₀	22.21±0.51bcd	19.52±0.81bcd	3.43±0.06de	2.72±0.1e	6713.93±168.76	6139.17±219.42	1770.71±71.90d	1683.46±40.52d	5.27±0.07d	4.96±0.12d
MC ₁₀₀	22.83±0.47bc	20.80±0.55ab	3.58±0.06cd	2.98±0.15d	6225.12±72.70	5349.7±194.18	1946.76±37.77c	1827.99±57.42c	5.38±0.18d	5.08±0.17cd
LSD	1.37*	1.67*	0.22*	0.24*	277.87^{NS}	327.27^{NS}	117.87*	108.79*	0.45*	0.47*
(N × MC)										

*Significant, ^{NS}non-significant. Data represent average of four replications± standard error. Different lowercase letters show significant differences ($P \leq 0.05$) among different treatments

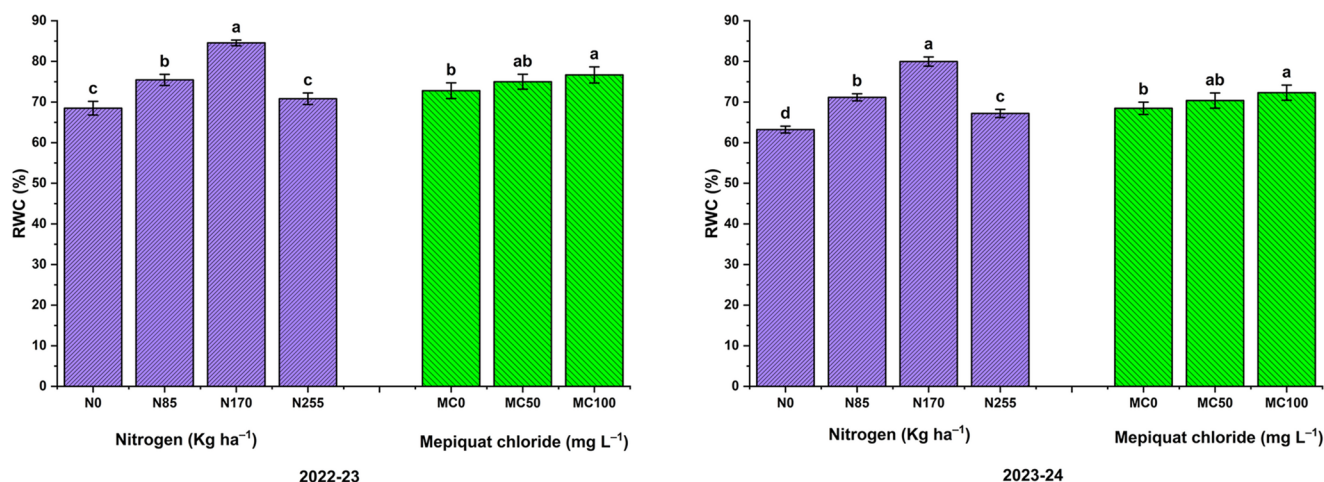


Fig. 2 Effect of nitrogen and mepiquat chloride (MC) application rates on relative water contents (RWC) of *Brassica napus* L. during 2022-23 and 2023-24. Different letter(s) on the bars indicate statistically sig-

nificant difference ($p \leq 0.05$) among treatments. Error bars indicate the standard error of the mean

year respectively in $N_0 + MC_{100}$ treated plants (Fig. 3C, D). The lowest CAT activity of 2.27 U mg^{-1} protein and 2.51 U mg^{-1} protein was observed in response to $N_{170} + MC_0$ treatments during 2022-23 and 2023-24, respectively (Fig. 3C, D).

Malondialdehyde ($\mu\text{mol g}^{-1}$)

During both years, the interactive effects of N and MC on malondialdehyde (MDA) contents was significant ($p < 0.05$). During the first year of experimentation, the MDA contents were the lowest in plants treated with $N_{170} + MC_{50}$ ($0.41 \mu\text{mol g}^{-1}$) (Fig. 3E, F) while the highest concentration of MDA was recorded in $N_0 + MC_{50}$. During the second year, nitrogen application @ 170 kg ha^{-1} in combination with MC_{50} or MC_{100} resulted in very low MDA contents; $0.61 \mu\text{mol g}^{-1}$ and $0.66 \mu\text{mol g}^{-1}$ respectively. Notably, the highest MDA contents were recorded in plants receiving only MC without nitrogen fertilization (Fig. 3E, F).

Lodging and shattering (%)

The interaction between N and MC on shattering and lodging was significant in both year ($P < 0.05$). Maximum lodging was observed in plants which received only nitrogen; however, lodging was significantly reduced when nitrogen application was supplemented with foliar application MC during both years (Fig. 4A, B). Amongst nitrogen treatments, 255 kg N ha^{-1} without MC caused the highest lodging, as compared to the plants deprived of nitrogen (Fig. 4A, B).

In the year 2022-23, the nitrogen application @ 170 kg ha^{-1} along with foliar application of MC @ 50 or 100 mg L^{-1} resulted in minimum shattering of 22.82% and 21.04% ,

respectively (Fig. 4C). In the second year of experimentation, nitrogen supply @ 85 – 170 kg ha^{-1} augmented with foliar application of MC @ 50 or 100 mg L^{-1} resulted in lower pod shattering (Fig. 4D). Control plants, receiving neither nitrogen nor MC or only 50 mg L^{-1} MC ($N_0 + MC_{50}$) were prone to highest shattering during both years (Fig. 4C, D). Overall, the results showed that shattering was significantly reduced depending on the treatments, whereby significantly lower shattering was observed in plants which received combined application of 170 kg N ha^{-1} supplemented with foliar application of either 50 or 100 ppm of MC (Fig. 4).

Nitrogen use efficiency

The interaction of nitrogen and MC on nitrogen use efficiency matrixes were significant during both years of study ($P < 0.05$) (Table 4) with following results:

During both years, an increase in nitrogen application from 85 kg ha^{-1} to 170 kg ha^{-1} without MC did not appreciably change nitrogen apparent recovery (NAR), however, further increase in nitrogen rate to 255 kg ha^{-1} resulted in decreased NAR. The recovery efficiency of applied nitrogen (N_{85} , N_{170} , N_{255}) was decreased with increasing concentration of MC. During first year of planting, the highest NAR of 53.68% was observed in $N_{170} + MC_{50}$ treated plants, while the lowest (41.86%) was observed in $N_{85} + MC_{100}$ treatment. Similar trend was observed during the following year as is evident from the data in Table 4.

Agronomic nitrogen use efficiency (AgE) was highly influenced by the combined application of N and MC during both years ($P < 0.05$). In 2022-23, the maximum AgE was recorded in $N_{170} + MC_{50}$, $N_{170} + MC_{100}$ and $N_{85} + MC_{50}$, treatments, while the lowest was noted in $N_{255} + MC_0$ treated

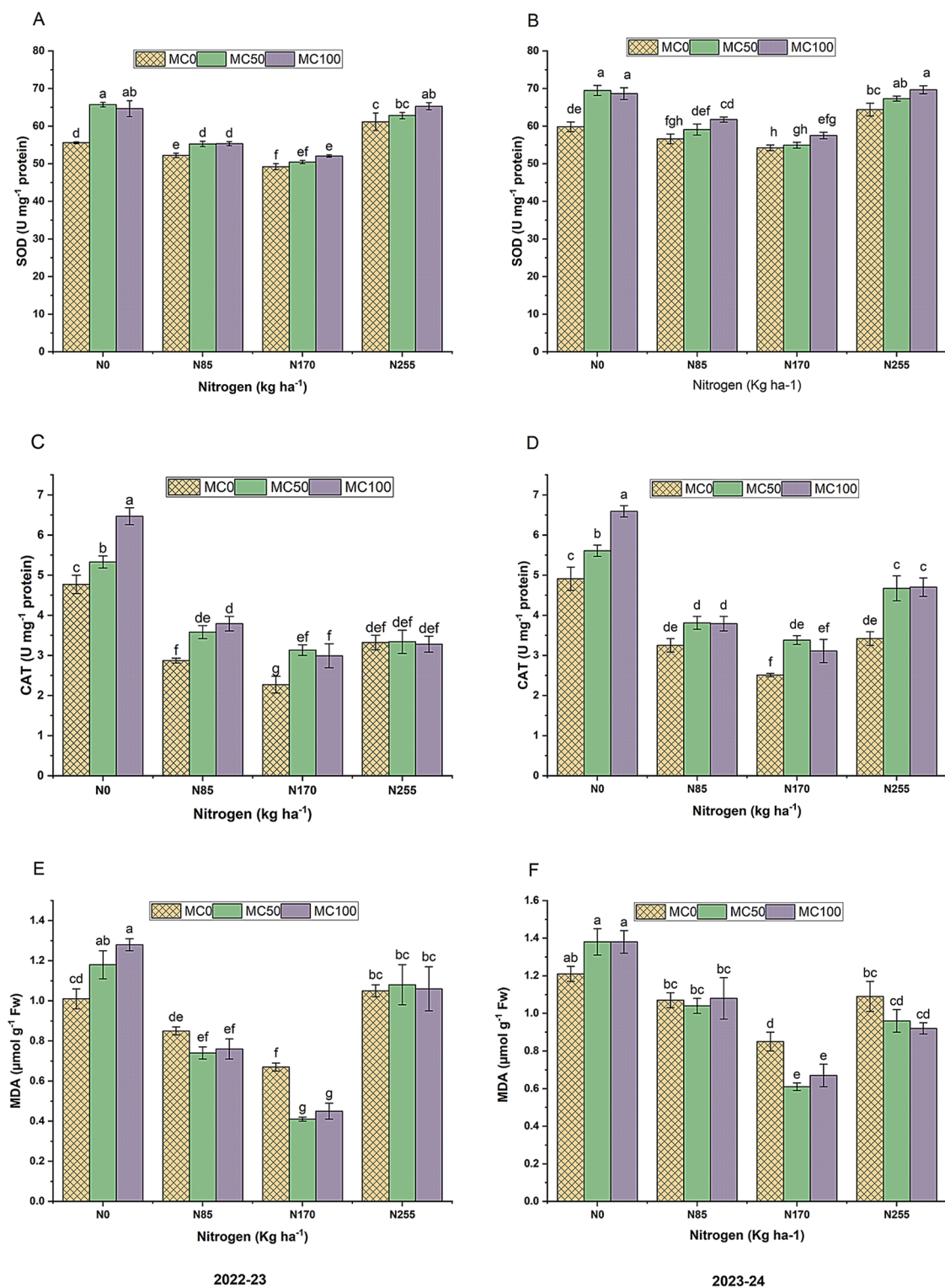


Fig. 3 Effect of nitrogen and mepiquat chloride (MC) application rates on catalase (CAT) and superoxide dismutase (SOD) activity and malondialdehyde (MDA) production in *Brassica napus* during 2022-23 and

2023-24. Different letter(s) on the bars indicate statistically significant difference ($p \leq 0.05$) among treatments. Error bars indicate the standard error of the mean

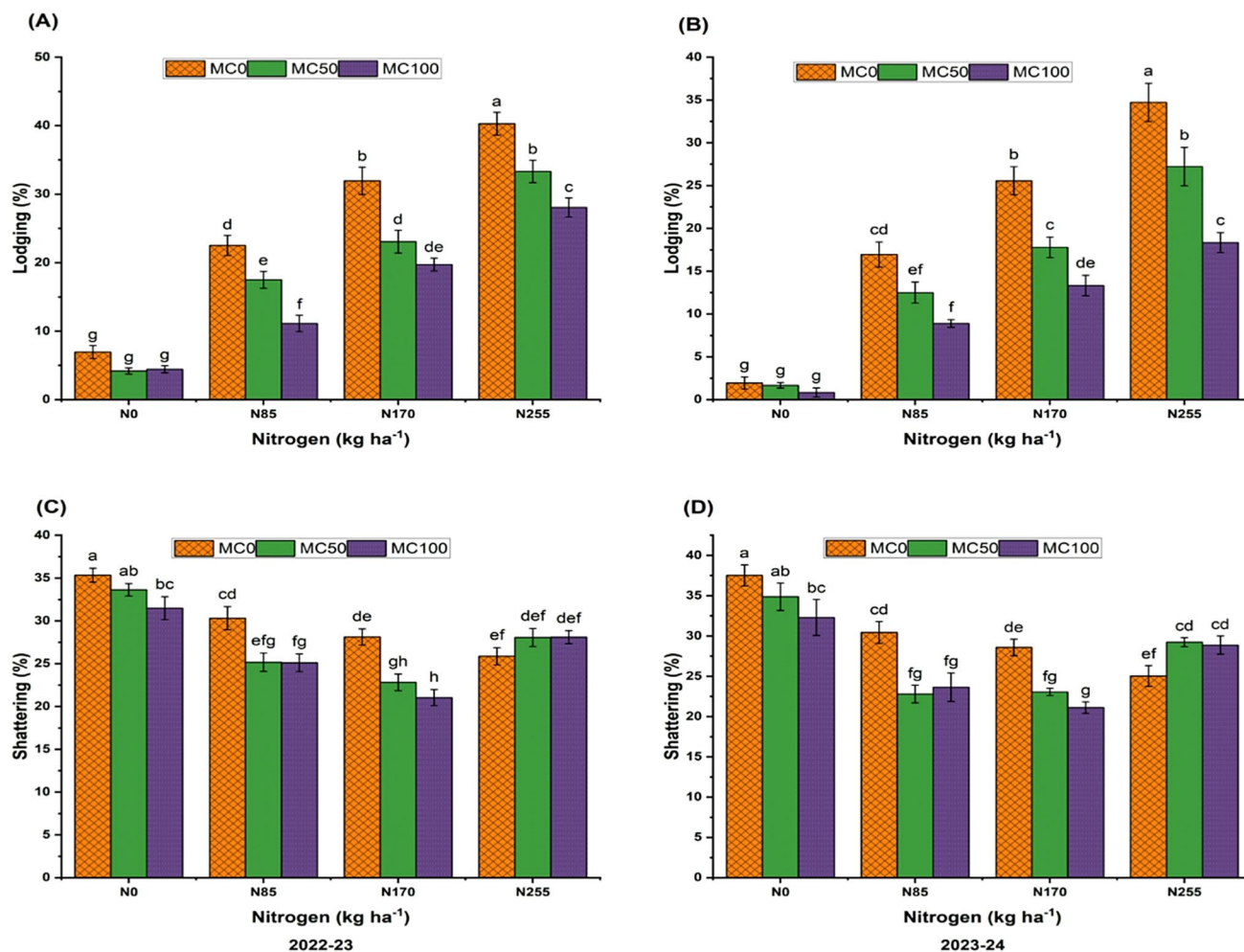


Fig. 4 Fig. 4: Effect of nitrogen and mepiquat chloride (MC) application rates on lodging and shattering of Brassica napus during 2022-23 and 2023-24. Different letter(s) on the bars indicate statistically significant difference ($p \leq 0.05$) among treatments

plants. In 2023-24, treatments comprising $N_{170} + MC_{100}$ and $N_{170} + MC_{50}$ resulted in significantly higher AgE against the (Table 4).

Nitrogen utilization efficiency (UTE) and agro-physiological efficiency (AgPE) decreased with increasing nitrogen application without MC and vice versa when nitrogen application was supplemented with MC. During both years, the highest UTE was recorded for nitrogen rate of 85 kg ha⁻¹ supplemented with 50 mg/L or 100 mg/L of MC. The highest AgPE was witnessed for N85 and N170 combined with 50 or 100 mg L⁻¹ of MC during 2022-23. While in the second year, the highest AgPE was noticed in N85 and N170 + MC100 treatments (Table 4). Partial factor productivity (PFP) of nitrogen fertilizer was decreased with increasing nitrogen during both years. However, when nitrogen was supplemented with MC, PFP was significantly improved. The highest PFP of 20.76 kg kg⁻¹ and 20.15 kg kg⁻¹ was observed when plants were treated with 85 kg ha⁻¹

supplemented with 50 mg L⁻¹ MC during 1st and 2nd year respectively (Table 4).

Correlation

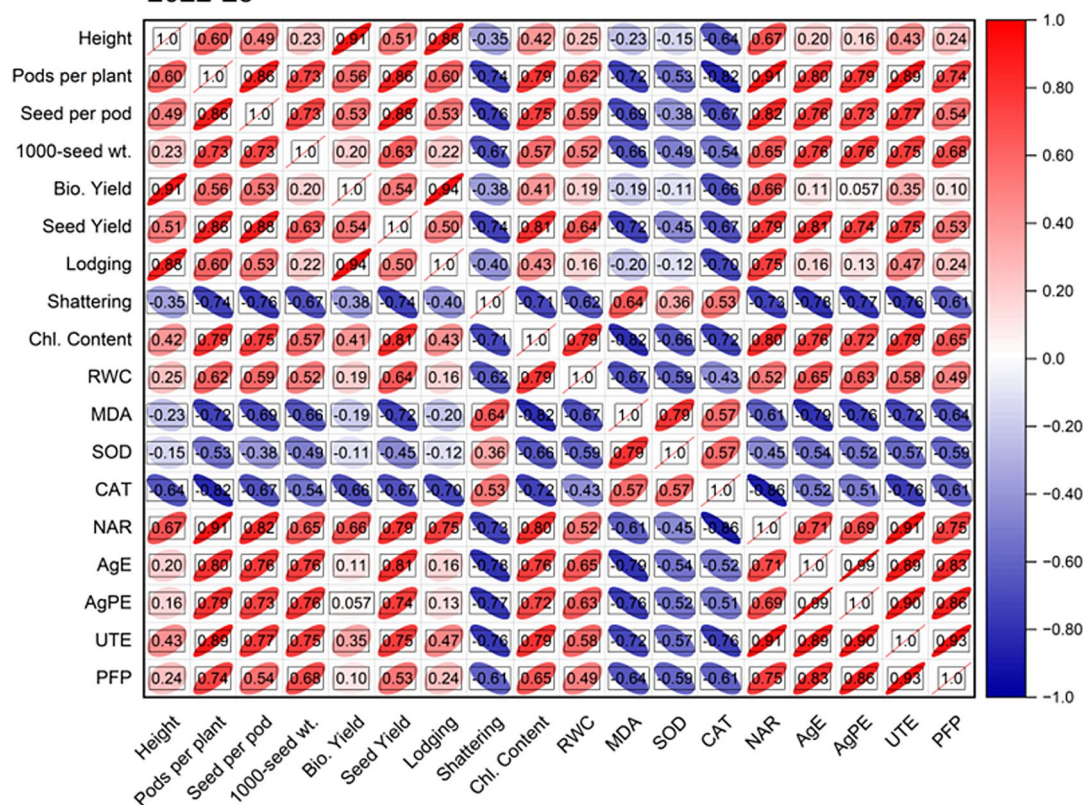
The Pearson correlation was employed to measure how strongly the variables were correlated with each other in response to nitrogen and mepiquat chloride application (Fig. 5). The heat map of Pearson correlation for different variables showed that in first and second year respectively, the seed yield was positively correlated with number of pods plant⁻¹ ($r=0.86, 0.91$), seeds pod⁻¹ ($r=0.88, 0.85$), 1000-seed weight ($r=0.63, 0.54$), chlorophyll contents ($r=0.61, 0.83$), relative water contents, RWC ($r=0.64, 0.78$) and nitrogen use efficiency matrixes; NAR ($r=0.79, 0.72$), AgE ($r=0.81, 0.79$), AgPE ($r=0.74, 0.73$), UTE ($r=0.75, 0.75$) and PFP ($r=0.53, 0.53$). However, the seed yield showed moderate to strong negative correlation with shattering ($r=-0.74, -0.64$) and lipid peroxidation (MDA) ($r=0.72, 0.86$).

Table 4 Nitrogen use efficiency matrices; nitrogen apparent recovery (NAR), agronomic efficiency (AgE), agro-physiological efficiency (AgPE), nitrogen utilization efficiency (UTE) and partial factor productivity (PPF) of *Brassica napus* L. influenced by nitrogen (N) and mepiquat chloride (MC)

	NAR (%)		AgE (kg kg ⁻¹)		UTE (kg kg ⁻¹)		AgPE (kg kg ⁻¹)		PPF (kg kg ⁻¹)	
	2022-23	2023-24	2022-23	2023-24	2022-23	2023-24	2022-23	2023-24	2022-23	2023-24
Nitrogen (kg ha⁻¹)										
N ₈₅	44.55b	44.15b	4.66a	4.17a	14.01a	14.06a	10.58a	9.83a	19.57a	19.23a
N ₁₇₀	50.14a	48.07a	4.83a	4.39a	12.60b	12.64b	9.62b	9.27a	12.29b	11.92b
N ₂₅₅	46.00b	40.81c	2.03b	1.58b	8.89c	9.29c	4.25c	4.19b	7.00c	6.61c
LSD value	1.97*	3.42*	0.43*	0.36*	0.46*	0.611*	0.94*	1.21*	0.47*	0.47*
Mepiquat chloride (mg L⁻¹)										
MC ₀	46.18b	47.92a	2.04b	1.62b	10.51b	10.30c	4.40c	3.33c	11.80c	11.72c
MC ₅₀	48.41a	46.02a	4.66a	4.10a	12.35a	12.32b	9.50b	8.74b	13.87a	13.35a
MC ₁₀₀	46.06b	39.09b	4.81a	4.42a	12.63a	13.37a	10.56a	11.22a	13.20b	12.69b
LSD value	1.97*	2.43*	0.43*	0.36*	0.46*	0.611*	0.94*	1.21*	0.47*	0.47*
Nitrogen × Mepiquat chloride (Interaction)										
N ₈₅ MC ₀	46.42 ± 1.93bc	50.48 ± 2.42a	2.93 ± 0.18c	2.77 ± 0.23c	12.95 ± 0.16c	13.03 ± 0.45c	6.36 ± 0.56b	5.57 ± 0.63 cd	18.88 ± 0.48b	19.30 ± 0.54b
MC ₅₀	45.37 ± 1.07 cd	44.33 ± 1.22c	5.7 ± 0.39ab	5.02 ± 0.18b	14.65 ± 0.19a	14.56 ± 0.64a	12.58 ± 0.85a	11.35 ± 1.14b	20.76 ± 0.22a	20.15 ± 0.24a
MC ₁₀₀	41.86 ± 0.80e	37.66 ± 0.70d	5.35 ± 0.12b	4.73 ± 0.12b	14.42 ± 0.33ab	14.59 ± 0.47a	12.81 ± 0.44a	12.56 ± 0.28ab	19.08 ± 0.44b	18.25 ± 0.38c
N ₁₇₀ MC ₀	49.46 ± 1.34b	48.71 ± 1.16ab	2.11 ± 0.32d	1.56 ± 0.13d	10.20 ± 0.43d	10.04 ± 0.22d	4.30 ± 0.69c	3.20 ± 0.23ef	10.08 ± 0.25e	9.82 ± 0.28e
MC ₅₀	53.68 ± 1.17a	51.57 ± 0.94a	6.36 ± 0.50a	5.73 ± 0.25a	13.64 ± 0.31bc	13.45 ± 0.2bc	11.83 ± 0.77a	11.14 ± 0.65b	13.89 ± 0.36c	13.30 ± 0.23d
MC ₁₀₀	47.27 ± 0.78bc	43.93 ± 1.08c	6.02 ± 0.44ab	5.89 ± 0.25a	13.95 ± 0.55ab	14.43 ± 0.25ab	12.72 ± 0.75a	13.46 ± 0.88a	12.89 ± 0.42d	12.65 ± 0.18d
N ₂₅₅ MC ₀	42.65 ± 1.2de	44.59 ± 1.65bc	1.11 ± 0.08e	0.55 ± 0.16e	8.39 ± 0.12f	7.84 ± 0.12f	2.54 ± 0.15d	1.23 ± 0.34f	6.42 ± 0.14 g	6.06 ± 0.13 g
MC ₅₀	46.17 ± 0.87bc	42.15 ± 1.32c	1.92 ± 0.37d	1.56 ± 0.24d	8.76 ± 0.32ef	8.95 ± 0.11e	4.08 ± 0.73 cd	3.72 ± 0.6de	6.94 ± 0.28 fg	6.60 ± 0.16 fg
MC ₁₀₀	49.05 ± 1.53b	35.68 ± 1.82d	3.06 ± 0.21c	2.66 ± 0.27c	9.52 ± 0.17de	11.07 ± 0.49d	6.15 ± 0.49b	7.62 ± 1.22c	7.64 ± 0.15f	7.17 ± 0.23f
LSD(N × MC)	3.42*	2.43*	0.75*	0.63*	0.79*	1.06*	1.64*	2.10*	0.80*	0.82*

*Significant, ^{NS}non-significant. Data represent average of four replications ± standard error. Different lowercase letters show significant differences ($P < 0.05$) among different treatments

2022-23



2023-24

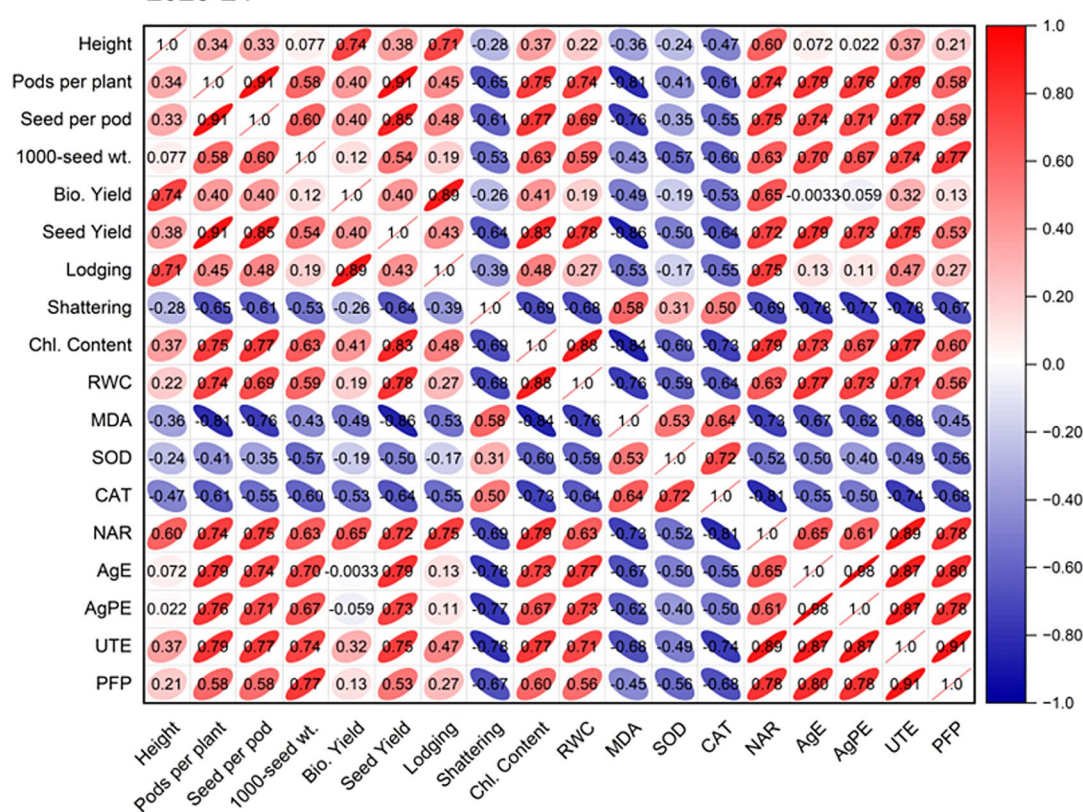


Fig. 5 Correlation among different variables in response to nitrogen and mepiquat chloride application rate during 2021-22 and 2023-24

A weak to moderate positive correlation was witnessed between shattering, antioxidant activity and lipid peroxidation, however, shattering was negatively correlated with NUE. Lodging showed a strong positive correlation with plant height ($r=0.85, 0.71$), biological yield ($r=0.94, 0.89$) and nitrogen uptake efficiency (NAR) ($r=0.75, 0.75$) but weak correlation with other nitrogen use efficiency indices (Fig. 5).

Discussion

Brassica napus is highly responsive to nitrogen fertilizer (Seepaul et al. 2019). Excessive use of nitrogen fertilizer results in profuse vegetative growth of plants, increases likelihood of lodging (Zhang et al. 2017), hinders mechanical harvesting, increases incidence of pod shattering which leads to high yield losses during harvesting. Present piece or work highlighted prospective use of nitrogen and mepiquat chloride on growth, yield and shattering of rainfed *B. napus*.

Higher N fertilizer rates generally produce taller plants while MC significantly reduces plant height (Boman and Westerman 1994). Application of MC prevents excessive growth and hyper elongation cell by arresting activity of gibberellic acid, diverts translocation of photosynthates towards stem and roots (Leal et al. 2020) which results in compact plants with stiff stem. However, overuse of mepiquat chloride is likely to overly inhibit plant growth which is manifested in the form of stunted plants. We noted an improvement in plant height, primary branches and reproductive branches (secondary branches) in response to nitrogen application which is supported by earlier studies (Seepaul et al. 2019; Yahbi et al. 2022, 2024). The shoot number and plant height is strongly influenced by nitrogen (N) supply (Luo et al. 2017) due to its role in cell elongation and multiplication. The combined nitrogen and mepiquat chloride led to a more balanced growth pattern, where the plant enjoys applied nitrogen, but with controlled architecture (Luo et al. 2020; Reddy et al. 1996). An ideal plant height is associated with more compact canopy which improves light penetration and increases photosynthesis (Tung et al. 2019).

The RWC is an indication of cells' water status and turgor pressure. Turgor pressure helps to maintain structure of the cell and plant organs including pods. The shattering resistance in *Brassica napus* is strongly associated with water content in pod walls (Kuai et al. 2016). Our results showed that optimum N and MC supply improved RWC but high nitrogen dose and MC limited leaf RWC. Nitrogen improves succulence of plants (Sharma et al. 2016).

However, an increase in N above optimum results in development of large canopy with larger leaf area. The greater leaf area transpires more water which depletes available soil moisture more rapidly and results in reduced RWC under limited moisture availability. Similarly, nitrogen application lowers leaf stomatal conductance in the early flowering stage (Zangani et al. 2021) and helped the plants to maintain higher RWC. Likewise, mepiquat chloride reduces leaf area and reduces transpiration water loss (Wang et al. 2024) and enables the plants to retain water more effectively, leading to higher RWC (Abdou et al. 2021; Fanaei et al. 2009).

Nitrogen is integral constituent of amino acids, needed for proteins and enzymes involved in the complex pathway of chlorophyll biosynthesis in plants (Novoa and Loomis 1981) (Barker and Pilbeam 2015; Novoa and Loomis 1981). Nitrogen application in *Brassica napus* is reported to increase leaf chlorophyll contents and quantum yield of photosystem II (Zangani et al. 2021). Whereas, mepiquat chloride controls plant development, optimizes uptake and the utilization of nitrogen, improves translocation of assimilates (Wang et al. 2024) and chlorophyll contents without abating photosynthesis (Rosolem et al. 2013). Therefore, integrated application of nitrogen and MC optimizes chlorophyll biosynthesis, increases photosynthetic efficiency, growth and yield traits which leads to higher yield (Leghari et al. 2016; Yadav et al. 2017).

B. napus is responsive to nitrogen fertilizer; however, seed yield increases only up to a certain level of nitrogen application rate (Yahbi et al. 2022). The optimum nitrogen for *B. napus* ranged from 90 to 240 kg N ha⁻¹ depending on site and cultivars (Rathke et al. 2006). Potential yield of *Brassica napus* is directly correlated with number of pods per plant and seeds per pods which are significantly affected by nitrogen application rate (Jehangir et al. 2024; Yahbi et al. 2022, 2024). The positive effect of nitrogen on the seed yield of oilseed crops like canola and oilseed rape has been intensively reported (Jehangir et al. 2024; Rathke et al. 2006). Nonetheless, some authors noted stagnation or even decline in seed yield at high rates of N fertilizer (Yahbi et al. 2022, 2024).

In *B. napus*, a significant amount of endogenous nitrogen is translocated from the leaves to the emerging inflorescence, supporting structures (Seepaul et al. 2019), the stems and to taproots which act as a buffering storage compartment later used to supply the reproductive tissue (Rossato et al. 2001). MC alters the source-sink relationship by increasing translocation and assimilation of photo-assimilates, nitrogen and other nutrients towards developing seeds which results in higher seed test weight (Gwathmey and Clement 2010; ZHAO et al. 2019) and final yield. Also, increasing nitrogen supply extends the seed filling duration by enhancing gas exchange (Zangani et al. 2021), resulting in healthier seeds

and ultimately higher seed yield. We noted that combined application of moderate to high levels of nitrogen and MC enhanced plant growth, yield and yield contributing traits of *B. napus*. However, individual excessive nitrogen and MC inputs imparted adverse effects (Tables 3 and 4). Our results are consistent with previous studies (Abbas et al. 2022), indicating that the application of MC and N improved pod setting, number of seeds per pod (Allen and Morgan 1972; Chapman et al. 1983) and to mitigate lodging (Tan and Temel 2005). Adequate nitrogen supply promotes vigorous vegetative growth, resulting in robust leaf area and higher biological yield (Boman and Westerman 1994; Cheema et al. 2009; Leal et al. 2020). Contrary, mepiquat chloride inhibits gibberellins biosynthesis, reduces internodal length and leads to shorter and sturdier plants with lower biological yield (Wang et al. 2014). Mepiquat chloride enables the plants to utilize nitrogen more effectively by promoting a more efficient plant architecture, and controlling excessive biomass of plant (Cheema et al. 2009). Therefore, application of MC has been frequently reported to improve yield of various crops (Kamran et al. 2018; Boman and Westerman 1994; Leal et al. 2020; Polat et al. 2017).

Nitrogen deficiency or toxicity induces oxidative stress in plants. Therefore, both deficiency and excess of nitrogen increases concentration of reactive oxygen species (ROS), which are responsible for lipid peroxidation of cell membranes (Yañez-Mansilla et al. 2015). The plants cope oxidative stress by activating antioxidant defense system. Nitrogen is a building block of antioxidants such as superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD) (Zhang et al. 2022; Severino and Oliveira 2024). These antioxidants help plants scavenge reactive oxygen species and enable them to achieve maximum yield even under stressful conditions. We found that the SOD and CAT activity was higher in plants facing nitrogen deficiency and provided with foliar application of MC (Fig. 3). It showed that nitrogen deficiency, when combined with MC, induces oxidative stress in plants, leading to activation of the antioxidant defense system. This is manifested by an increase in the activity of superoxide dismutase (SOD) and catalase (CAT). Singh et al. (2018) reported that nitrogen deficiency in plants induces oxidative stress (increased concentration of reactive oxygen species like H_2O_2 content) in plants and plants scavenge ROS by increasing activity of antioxidants. However, excessive nitrogen can lead to decreased SOD activity (Shehab and Guo 2021). Therefore, balance between MC and nitrogen application improves the SOD activity in the plant (Sun et al. 2022; Zhang et al. 2022). Our findings are supported by earlier studies, showing that prudent application of N and MC improves CAT activity in plant (Hou et al. 2024; Zhang et al. 2022). Ding et al. (2017) studied that, mepiquat chloride boosted antioxidant enzyme

activity by lowering biomolecule degradation. The reduced doses of nitrogen only also depicted lower SOD and CAT activity (Fig. 3). Adequate nitrogen supply produces more catalase, enhancing their ability to scavenge hydrogen peroxide (Polesskaya et al. 2004; Zervoudakis et al. 2015).

Oxidative stress is responsible for the peroxidation of polyunsaturated fatty acids in plant membranes, resulting in the production of Malondialdehyde (MDA). The lipid peroxidation is therefore considered a physiological indicator of stress responses (the higher MDA content in a plant the more will be peroxidation), and is often used as a biomarker to assess stress-induced cell damage or death in response to abiotic stress (Awasthi et al. 2018; Illés et al. 2021).

The nitrogen application rate had a significant effect on the measurable MDA contents of plants. For example, Illés et al. (2021) witnessed non-significant difference in the MDA contents in response to 0 kg N ha^{-1} and the 120 N ha^{-1} , but the high nutrient level of 300 N ha^{-1} significantly increased lipid peroxidation. Inadequate or excessive nitrogen at especially at reproductive growth stages causes significant lipid peroxidation of biomembranes (Illés et al. 2021; Kong et al. 2017). We noted higher MDA content in response to N_0 (nitrogen deficiency) coupled with MC (Fig. 3E, F) which is in agreement with previous studies (Kong et al. 2017). However, N_{170} combined with MC demonstrated lower MDA contents (Fig. 3E, F), showing that combined application of nitrogen and MC reduces likelihood of lipid peroxidation of biomembranes. Tian et al. (2021) reported that correct nitrogen fertilization decreases lipid peroxidation (decrease MDA content) in plants by improving nitrogen metabolism and photosynthetic efficiency coupled with improved antioxidant defense mechanism. Wang et al. (2024) revealed that application of MC resulted in a reduction in lipid peroxidation by scavenging reactive oxygen species (ROS) and lowering the concentrations of malondialdehyde (MDA) in plants. The role of nitrogen and MC to reduce the MDA contents is also reported by dos Santos et al. (2021) and (Saneoka et al. 2004). It indicated that harmonized use of nitrogen and MC is important strategy to improve antioxidant and mitigate lipid peroxidation.

Pod shattering is a key factor affecting the mechanical harvesting of oilseed rape (Qing et al. 2021; Kuai et al. 2016). Nitrogen promotes vegetative growth and plant height while MC impedes plant height (Boman and Westerman 1994). The plant height affects the structural integrity of the pods. The taller plants have more cylindrical and fragile stem which is liable to breaking or bending and had increased susceptibility to pod shattering (Qing et al. 2021) during mechanical harvesting. We noted that nitrogen deficiency resulted in higher pod shattering but reduced lodging, while MC application tended to reduce both shattering and lodging (Fig. 4) Excessive nitrogen supply results in

an imbalance between reproductive and vegetative growth, poor lignification in the sclerenchyma cells in the mechanical tissues and vascular bundles, makes the plants prone to structural damage and increases incidence of lodging (Zhang et al. 2016).

Both excessive and scanty nitrogen are responsible for weaker (Goyal and Huffaker 1984; Li et al. 2024; Peng et al. 2024) and thinner pod walls, which are less resilient to environmental stress making them susceptible to shattering (Kuai et al. 2023). Contrary, a rational nitrogen supply promotes healthy growth and strong stems, which decreases chance of lodging and pod shattering (Yahaya et al. 2023). Also, the water content in pod walls (Kuai et al. 2016), 1000-seed weight, pod thickness and volume, and plant branch height are significantly correlated with pod shattering resistance (Qing et al. 2021).

MC improves fruit retention and hastens maturity (Chalisse et al. 2022), increases lodging resistance by increasing stem physical strength, stem diameter and dry weight, and improved lignin biosynthesis (Kamran et al. 2018; Tan and Temel 2005). Stronger stems are less vulnerable to bend or break under the weight of heavy seed heads or adverse weather conditions such as wind and rain. Therefore, MC has been extensively employed to improving structural integrity of plants, modulate plant height, fruit retention, fruit branch length and to develop more compact plant canopy, and to improve shattering and lodging resistance and higher harvest efficiency (Qureshi et al. 2024; Li et al. 2024). However, overuse of mepiquat chloride is likely to overly inhibit plant growth (Cook and Kennedy 2000). Therefore, prudent nitrogen application in combination with plant growth regulators (MC) is anticipated to improve yield and harvest efficiency of *B. napus*.

Nitrogen accounts for the largest energy input in production of *B. napus*, but the crop is characterized by low N-use efficiency (Bouchet et al. 2016). Nitrogen uptake efficiency refers to how effectively roots absorb nitrogen from the soil as they grow and spread. Plants have a limited capacity to absorb and utilize applied nitrogen; excessive nitrogen is not taken up by the plants and is lost as ammonia volatilization and nitrate leaching. It leads to diminished returns in terms of yield output per unit of nitrogen applied i.e. low NUE (Ghadirnezhad Shiade et al. 2024; Vaziritabar et al. 2024).

Higher nitrogen supply has a negative relationship with nitrogen uptake efficiency (Fernando and Sparkes 2020). Therefore, increasing nitrogen supply increases recovery and utilization of nitrogen, whereas greater rates upsurge utilization but not recovery and agronomic efficiency in *B. napus* (Seepaul et al. 2019). The high nitrogen levels promote rapid vegetative growth and results in tall and weak plants, and make them susceptible to lodging. The root

system of lodged plants is damaged which limits nitrogen uptake efficiency (Ahmad et al. 2023).

The nitrogen management strategies could influence NUE and productivity oilseed rape. Application of N above the optimum rate reduces NUE in *B. napus* (Yahbi et al. 2024). For example, nitrogen apparent recovery (NAR) of 52.25% has been reported for nitrogen rate of 50 kg ha⁻¹, which declined to 32.16 by increasing nitrogen application rate to 150 kg ha with various fertilizer management practices (Hasanalideh and Hojati 2012). The agronomic nitrogen efficiency of 7.64 kg kg⁻¹, 10.96 kg kg⁻¹ and 10.13 kg kg⁻¹ has been reported for nitrogen application rate of 60, 80 and 100 kg kg⁻¹ in *Brassica rapa* (Jehangir et al. 2024). Similarly, agronomic nitrogen use efficiency of 14.89 kg kg⁻¹, NHI of 59.43 kg kg⁻¹ and physiological nitrogen use efficiency of 39.04 kg kg⁻¹ has been reported for *B. napus* with various integrated nutrient management practices (Hasanalideh and Hojati 2012).

The plant dry biomass and N uptake, irrespective of N levels, is positively correlated with root size (Wang et al. 2017). Therefore, remobilization of N from the shoot to roots by high shoot glutamine synthetase activity enhances root total soluble protein, which improves root growth for N uptake and NUE (Iqbal et al. 2020). MC arrests excessive vegetative growth and diverts translocation of resources towards reproductive organs (Fageria and Baligar 2005; Raun and Johnson 1999) and roots; and thereby improves root system, nitrogen uptake (Dong et al. 2023) and agronomic nitrogen use efficiency of applied fertilizer.

Synchronized distribution/translocation of nitrogen from source to sink is crucial for improved NUE, seed development and crop yield (Liang et al. 2023). For example, efficient source to sink remobilization of amino acids can improve NUE, pods per plant, number of seeds per plant and seed test weight yield and harvest index of oilseed rape (Liang et al. 2023). We noted that increasing MC doses declined NAR especially for N₈₅ which seemed to be correlated with reduced biomass yield; since, NAR is multiplicative output of nitrogen concentration in above ground biomass and dry matter yield. However, mepiquat chloride enhanced agronomic, utilization physiological efficiency (Table 4) by redirecting resources from excessive vegetative growth to reproductive organs, enhanced photosynthesis and nitrogen assimilation (Reddy et al. 1996; Tung et al. 2019). The application of mepiquat chloride is also reported to increase K uptake and use efficiency (partial factor productivity, agronomic efficiency, and apparent recovery efficiency) (Yang et al. 2014) which also contributed to seed yield. The improved seed productivity led to increased agronomic efficiency, nitrogen recovery, utilization and partial factor productivity in Brassica (Seepaul et al. 2019).

Conclusion

Sole application of nitrogen results in excessive vegetative growth, increases the incidence of lodging and shattering in *B. napus* causing significant yield losses. Nitrogen application supplemented with mepiquat chloride (MC), significantly halted excessive vegetative growth and plant height, manipulated plant architecture and increased nitrogen use efficiency (NUE). Nitrogen application at 85 kg ha⁻¹ combined with of 50 mg L⁻¹ of MC at rosette plus silique formation stage (12.5 g ha⁻¹ + 12.5 g ha⁻¹) is recommended to harvest maximum yield of *Brassica napus* genotype under consideration. However, nitrogen and MC application rate may vary for various genotypes depending on soil and environmental conditions and should be optimized. To the best of our knowledge, this is the first report which highlighted the integrated role of nitrogen and mepiquat chloride to mitigate lodging, shattering, improve NUE and yield of *B. napus*. Further research is suggested to investigate the effect of nitrogen × MC × harvest maturity on pod wall composition, shattering and yield of *B. napus*.

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