

## Effects of sowing time on fibre yield and chemical composition of flax (*Linum usitatissimum* L.) genotypes

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### Abstract

Flax is an important industrial crop known for its high-quality fibres and various applications in textiles, composites, pulp and paper, and other industries. This study investigates the impact of sowing time and phenological stages on fibre yield and chemical composition of different flax genotypes. Four genotypes viz. BARI Tishi-1, BD-10708, Canada, and China, were grown under various sowing dates viz. viz. 1st and 16th November, and 1st December. Fibre yield, cellulose, lignin, hemicellulose and extractives were measured. Sowing on November 16 produced the highest fibre yield, with early sowing leading to reduced yield. Analysis of chemical composition at flowering and post-flowering stages revealed significant variation in cellulose, lignin, hemicellulose, and extractive contents across genotypes. BARI Tishi-1 and China genotype demonstrated high and stable  $\alpha$ -cellulose levels (53–60%), particularly in later plantings and post-flowering stages. However, genotype Canada had high extractive (8–10%) and hemicellulose contents (21–24%). The present findings underscore the importance of optimizing sowing time to maximize fibre yield and quality, which is essential for meeting industrial standards and improving the economic value of flax.

**Keywords:** Flax;  $\alpha$ -Cellulose; Lignin; BARI Tishi-1; BD-10708; Canada; China; genotypes

### Introduction

Plant fibres, one of the oldest natural materials in human history, have been used for many purposes e.g., hunting rope or yarn making, fishing, netting, climbing, carrying, and textile weaving (Kılınc *et al.* 2017). In the beginning history of plant fiber use, several wild species were the main sources of raw materials. After the emergence of agriculture, people started to cultivate fibre yielding plants. The earliest evidence of using wild flax as a textile commodity dates back to 30,000 years ago and the first domesticated flax (*Linum usitatissimum* L., Fam.: Linaceae) in the Fertile Crescent region by c. 9,000 years ago (Anon., 2024). Fibers originating from phloem/bast of flax are naturally smooth, straight, and two to three times as strong as cotton fibres, and are used

for existing high-value markets in the textile, composites, paper/pulp, and other industrial uses (Hamilton, 1986).

Flax fibre stands out as the key member of the bast family for composite reinforcement because of its distinctive properties (Yan *et al.* 2014). High-quality cellulosic pulp is produced using various chemical processes, such as soda, kraft, and gas discharge plasma in water and electrolyte solutions (Gutiérrez and del Río, 2003; Rencoret *et al.* 2013; Titov *et al.* 2015). The high fibre length and aspect ratio make it perfect for creating high-strength paper, thus ensuring the production of banknotes with excellent mechanical strength (Bárta *et al.* 2023).

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This fibre is highly suitable for producing top-quality nanocellulose due to its high aspect ratio (Barbash *et al.* 2017).

In response to environmental concerns, natural fibres such as flax, cotton, and jute are highly sustainable composite reinforcement materials due to their ecological properties, low specific gravity, renewability, biodegradability, recyclability, and high mechanical properties. Among these options, flax is the most potent natural fibre used in reinforcing polymer-based composites (Moudood *et al.* 2019). The flax fibres have a density of  $1.5 \text{ g cm}^{-3}$  and an aspect ratio of 700-2000. They also demonstrate excellent mechanical properties, with a tensile strength of about 1500 MPa and a stiffness of around 90 GPa (Charlet *et al.* 2009). Using natural fibres to reinforce materials makes them lighter and stronger (Le Duigou and Baley, 2014). Flax accounted for approximately 50% of the total natural fibre used to reinforce composites (Barth and Carus, 2015). The flax fibre enhances the thermal stability of the polymer composite significantly (Malik *et al.* 2022; Mishra *et al.* 2020). Velde and Kiekens (2001) studied the physical, thermal, and mechanical properties of 12 flax fibre-reinforced polymer composites, which included low-density polyethylene (LDPE) and high-density polyethylene (HDPE). The mechanical strength of LDPE and HDPE composites increases as the fibre content increases (Li *et al.* 2009). Flax is considered a high-quality textile fibre, along with cotton, wool, and silk (Muzyczek, 2020). Flax fibre has demonstrated high performance in textile reinforcement of natural fibre composite systems (Goutianos *et al.* 2006). The chemical composition and physical properties of flax fibre determine its potential applications (Rahman *et al.* 2023).

Global climate change increases the occurrence of climate-related extremes e.g., numerous threats of extreme (high or low) temperature, cyclone and storm surge, inundation, salinity intrusion, and erratic rainfall with both increasing intensity and frequency, those are the major sources of variation in crop production. Drought stress and high temperature are important environmental factors, which restrict plant growth and often alter plant chemical composition, which in turn can affect (food, fodder, and fibre) quality, and decomposition rates (AbdElgawad *et al.* 2014). Climatic variations also influence the composition of natural fibres (Komuraiah *et al.* 2014). The variations in climatic conditions especially the temperature gradient influenced the growth, phenology and seed yield of flax cultivars (Sarwar *et al.* 2022). This study investigated the effects of sowing time and genotypes on the yield, physical characteristics and chemical makeup, including cellulose, hemicellulose, lignin, and extractive content of flax fibres.

## Materials and methods

The present experiment was carried out at Shoreartol, Alamnagar, Rangpur, Bangladesh under AEZ-3 from November 2019 to March 2020. In the experimental site, factorial trial was laid out in a split-plot design having three replications. Three sowing dates, viz. 1<sup>st</sup> and 16<sup>th</sup> November, and 1<sup>st</sup> December, respectively were followed in the main plot. Four genotypes of flax, viz. BARI Tishi-1, BD-10708, Canada and China, were selected based on their performance test in the previous experiments and used in the subplots. The individual plot size was  $3.0 \times 1.5 \text{ m}$  (10 lines) with continuous sowing. Seeds were sown at a depth of 2 cm in the soil with the help of a hand row. Recommended doses of fertilizer and manures were applied (BARC, 2018). Intercultural operations were done as and when necessary. Seeds of flax genotypes were sown on 1st and 16th November, and 1st December 2019. Each subplot was made sub-subplots and was cut three times viz. pre-flowering, 50% flowering, and post-flowering stages. The crop was harvested by March 2020 and the fibre yield ( $\text{gm}^{-2}$ ) was recorded. However, the chemical composition of fibres was determined in the laboratory following standard procedures.

### Toluene-ethanol extractive

The toluene-ethanol solubility of flax was determined according to the Tappi test method T 204 cm-97. 2 g of oven-dried chopped flax fibre (4-5 mm) was used to determine extractives. A solvent system consisting of toluene and ethanol in a 7:3 ratio was employed for the extraction process. The sample was placed in an extraction thimble, which was then inserted into a clean and dry Soxhlet extraction flask. Accurately measured 200 ml of toluene-ethanol solvent was added to the extraction flask, and the heater was adjusted to achieve a boiling rate that needed 12-15 minutes per cycle. The extraction process was carried out for 6 h. Upon completion of the extraction, the solvent was partially evaporated until reaching a volume of 20-25 ml. Subsequently, the remaining solution was transferred to a pre-weighed crucible and dried to a constant weight at  $105^\circ\text{C}$ . The amount of extractive was determined using the following equation:

$$\text{Extractive \%} = \frac{A}{W} \times 100$$

Where A is the weight of extract; W is the weight of flax

### Hemicellulose

Hemicellulose was determined by deducting  $\alpha$ -cellulose from holocellulose. Holocellulose was determined by

treating extractive-free flax fibre with  $\text{NaClO}_2$  solution (Sarkar *et al.* 2021). Approximately, 2 g of extractives free flax sample was taken to a 250 ml conical flask containing a volume of 100 ml of 3.5% sodium chlorite solution. The pH of the solution was maintained at 4 by adding  $\text{CH}_3\text{COOH}-\text{CH}_3\text{COONa}$  buffer. The flask closed with a lid was partially immersed in a hot water bath at 70-80°C for 4 h. The procedure was carried out in a fume hood to avoid toxic chlorine dioxide fumes due to the chemical reactions. After 4 h of heating, the flask was cooled under tap water. Next, the sample was filtered through a pre-weighed G2 sintered glass crucible very well with distilled water. The glass crucible along with the sample was kept in the drier at 105°C till the constant mass was achieved.

$$\text{Holocellulose, \%} = \frac{A}{W} \times 100$$

Where  $A$  = weight of holocellulose;  $W$  = weight of the sample  
Hemicellulose = (Holocellulose - ( $\alpha$ -cellulose) %

#### *$\alpha$ -Cellulose*

$\alpha$ -Cellulose was determined for the holocellulose following Tappi test method of T 203 cm-99. Each test involved taking approximately 1 g of oven-dried holocellulose and placing it in a 250 ml beaker. Then, 100 ml of 17.5% NaOH solution was gradually added to the sample. The mixture was gently stirred until completely dispersed and left to stand for 30 minutes. After that, 100 ml of distilled water was added to the mixture and it was stirred thoroughly with a glass rod. The mixture was then left to stand for another 30 minutes. After a total of 60 minutes, the filtrate was collected in another clean beaker. Then in a 250 ml flask 25 ml of the filtrate and 10.0 ml of 0.5N potassium dichromate solution were pipetted, to which 50 ml of concentrated sulfuric acid was added. After waiting for 15 minutes, 50 ml of distilled water was added, and the solution was cooled to room temperature. The solution was then titrated with 0.1N ferrous ammonium sulfate solution using a ferroin indicator. Additionally, a blank test was performed using 12.5 ml of 17.5% NaOH and 12.5 ml of distilled water.

$$\alpha - \text{cellulose, \%} = 100 - \frac{137(V_2 - V_1) \times N}{A \times W}$$

Where,  $V_1$  = titrant volume for the sample titration,  $V_2$  = titrant for blank titration,  $N$  = normality of the ferrous ammonium sulfate solution,  $A$  = volume of the filtrate used in the oxidation,  $W$  = oven-dry weight of the sample

#### *Lignin*

Lignin content in the sample was determined according to the Tappi test method T 222 om-02 with some modification. An extractive-free 1 g flax sample was placed in a 100 ml beaker. Then 15 ml of 72% sulfuric acid was slowly added to the beaker while the sample was stirred with a glass rod. The mixture was left for 2 h with intermittent stirring every 10-15 min to ensure complete hydrolysis. Thereafter, the mixture was transferred to a 1000 ml conical flask containing 300 ml of distilled water. The total volume was adjusted to 575 ml. The flask was then autoclaved for 60 min and left overnight for the insoluble lignin to settle. The precipitated lignin was quantitatively separated by filtering through a grade 4 sintered glass crucible and then dried to a constant weight at 105°C.

$$\text{Lignin, \%} = \frac{A}{W} \times 100$$

Where  $A$  = weight of lignin;  $W$  = weight of the sample

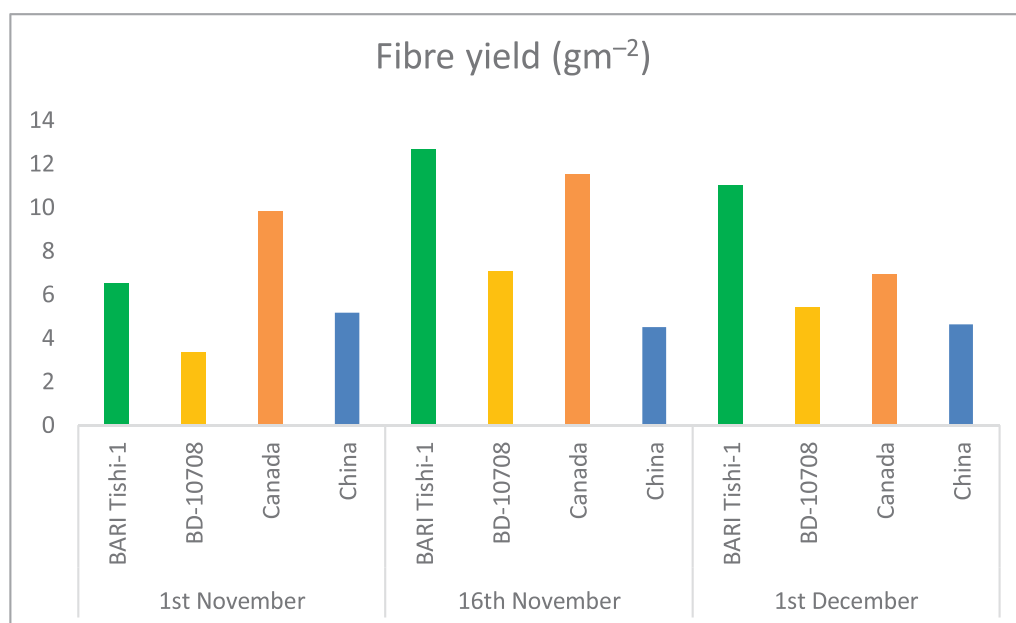
#### *Data analysis*

Data were analyzed statistically following the analysis of variance (ANOVA) technique, using Statistix 10 software package and means were separated by the Least Significant Difference test (LSD) at 5% level of significance.

### **Results and discussion**

#### *Effect of sowing dates on fibre yield*

The flax fibre yield varied with both the genotypes and sowing dates. Among the sowing dates, the 16<sup>th</sup> November sowing resulted in the highest fibre yield ( $\text{gm}^{-2}$ ) compared to the others (Figure 1). The lower fibre yield in early sowing might be due to the higher soil moisture and soil temperature hampering initial plant establishment through enhancing the collar rot disease which ultimately retards the fibre production and yield. Moreover, plants sown in early November were exposed to cold temperatures (vernalization) which induces (early) flowering with shortened vegetative growth. On the contrary, the lower yield of 1<sup>st</sup> December sowing might be due to the delayed germination in low soil temperature and subsequent climatic conditions lowering growth and fibre yield as well (Sarwar *et al.* 2022). Among the genotypes, BARI Tishi-1 was the higher yielder which might be due to its adaptability in the local environment. However, the genotype Canada started with a significantly higher yield in early sowing (Figure 1).



**Fig. 1. Fibre yield of flax genotypes**

#### *Chemical composition of flax genotypes at different phenological stages*

Cellulose, lignin, and hemicellulose are key components of lignocellulosic fibre, with cellulose being the most important due to its strength, durability, and versatility. Bast fibre, derived from plants like jute, kenaf, and hemp, is highly valued for its high cellulose and low lignin contents (Panigrahy *et al.* 2006). Table I displays the chemical composition of four different flax genotypes harvested at the flowering and

post-flowering stages. The statistical analysis revealed significant variations in chemical composition among genotypes harvested at the same time, underscoring the influence of genetic factors on fibre quality (Rahman *et al.* 2023).

Extractive content varies among genotypes and between stages (Table I). Extractives tend to decrease slightly from flowering to post-flowering stages for most genotypes except BD-10708, where extractives increase from 7.03-8.08%. The Canada genotype has the highest extractive content at flower-

**Table I. Phenological stages on chemical composition of flax genotypes**

Genotype	Stage	Extractive materials (%)	Lignin (%)	$\alpha$ Cellulose (%)	Hemicellulose (%)
BARI Tishi-1	Flowering	8.62±0.45	7.61±0.30	57.99±2.29	15.99±0.63
	Post-Flowering	7.79±0.31	7.71±0.30	59.55±2.29	17.14±1.08
BD-10708	Flowering	7.03±0.28	6.75±0.27	61.06±2.43	14.94±0.59
	Post-Flowering	8.08±0.38	11.03±0.44	51.51±3.03	17.26±0.68
Canada	Flowering	9.93±0.39	7.60±0.30	50.70±2.00	23.99±0.95
	Post-Flowering	7.51±0.30	7.37±0.29	51.75±2.54	21.32±0.84
China	Flowering	7.05±0.28	8.40±0.33	53.70±2.12	19.41±0.97
	Post-Flowering	7.36±0.29	6.73±0.27	59.38±2.34	19.14±0.76

ing (9.93%), indicating potentially more resin, oil, or other non-structural components at this stage compared to other genotypes.  $\alpha$ -Cellulose content increases slightly in BARI Tishi-1 and China genotype from flowering to post-flowering stages, with the highest values observed in BD-10708 at flowering (61.06%). BD-10708 displays a noticeable decline in  $\alpha$ -cellulose from flowering to post-flowering (Table I). Hemicellulose content appears variable, with Canada genotype showing a high proportion at both stages, particularly during flowering (23.99%). This could make it less important for fibre extraction. For BARI Tishi-1 and BD-10708, hemicellulose content increases in the post-flowering, while for China genotype, there is minimal change, suggesting stability in hemicellulose composition in this genotype. Komuraiah *et al.* (2014) reported that hemicelluloses are positively correlated with specific Young's modulus, specific strength, diameter, and moisture gain, and negatively correlated with tensile strength, density, length microfibril angle, and failure

strain. Lignin content is crucial for strength and rigidity. Most genotypes show minor changes in lignin content across stages, except for BD-10708, where lignin rises significantly from 6.75-11.03% at post-flowering. This increase may impact the material's rigidity and resistance to degradation. The China genotype shows a reduction in lignin from flowering (8.40%) to post-flowering (6.73%), suggesting a potential reduction in structural stiffness. The tensile strength, specific strength, specific Young's modulus, density, diameter, and length of fibres are negatively, and the microfibril angle, failure strain, and moisture gain are positively correlated with lignin content (Komuraiah *et al.* 2014).

BARI Tishi-1 and China genotypes exhibit stability in cellulose and hemicellulose contents across stages, making them potential candidates for applications requiring high cellulose content. The aforementioned changes indicate that the chemical contents of the genotypes are strongly influenced by growth phases.

**Table II. Planting time and phenological stages on chemical composition of flax genotypes**

Accession	Planting time and stage*	Extractive materials (%)	Lignin (%)	$\alpha$ -Cellulose (%)	Hemicellulose (%)
BARI Tishi-1	1 <sup>st</sup> planting	4.31 $\pm$ 0.17	6.94 $\pm$ 0.47	52.36 $\pm$ 2.07	17.75 $\pm$ 0.70
	2 <sup>nd</sup> planting	2.74 $\pm$ 0.11	7.07 $\pm$ 0.28	56.61 $\pm$ 2.23	17.36 $\pm$ 0.69
	3 <sup>rd</sup> planting (PrFS)	5.82 $\pm$ 0.23	6.22 $\pm$ 0.25	57.93 $\pm$ 2.29	18.03 $\pm$ 0.71
	3 <sup>rd</sup> planting (PoFS)	11.03 $\pm$ 0.44	7.65 $\pm$ 0.30	61.62 $\pm$ 2.43	20.04 $\pm$ 0.79
BD-10708	1 <sup>st</sup> planting	10.55 $\pm$ 0.42	7.98 $\pm$ 0.51	48.84 $\pm$ 1.93	17.39 $\pm$ 0.69
	2 <sup>nd</sup> planting	6.92 $\pm$ 0.27	7.61 $\pm$ 0.30	49.91 $\pm$ 1.97	15.15 $\pm$ 0.60
	3 <sup>rd</sup> planting (PrFS)	6.27 $\pm$ 0.25	3.43 $\pm$ 0.14	55.00 $\pm$ 2.17	12.57 $\pm$ 0.50
	3 <sup>rd</sup> planting (PoFS)	6.87 $\pm$ 0.27	13.18 $\pm$ 1.52	64.34 $\pm$ 2.54	17.87 $\pm$ 0.70
Canada	1 <sup>st</sup> planting	10.11 $\pm$ 0.40	10.00 $\pm$ 0.93	49.03 $\pm$ 1.93	13.70 $\pm$ 0.54
	2 <sup>nd</sup> planting	9.43 $\pm$ 0.37	6.90 $\pm$ 0.27	48.36 $\pm$ 1.91	17.42 $\pm$ 0.69
	3 <sup>rd</sup> planting (PrFS)	10.48 $\pm$ 0.41	8.79 $\pm$ 0.35	47.67 $\pm$ 1.88	34.23 $\pm$ 1.35
	3 <sup>rd</sup> planting (PoFS)	9.69 $\pm$ 0.38	11.34 $\pm$ 0.65	50.13 $\pm$ 1.98	29.42 $\pm$ 1.16
China	1 <sup>st</sup> planting	6.50 $\pm$ 0.26	8.09 $\pm$ 0.32	56.83 $\pm$ 2.54	15.69 $\pm$ 0.62
	2 <sup>nd</sup> planting	4.96 $\pm$ 0.20	9.17 $\pm$ 0.36	60.06 $\pm$ 2.37	16.36 $\pm$ 0.65
	3 <sup>rd</sup> planting (PrFS)	8.41 $\pm$ 0.33	8.31 $\pm$ 0.33	60.61 $\pm$ 1.39	17.13 $\pm$ 0.68
	3 <sup>rd</sup> planting (PoFS)	6.26 $\pm$ 0.25	9.45 $\pm$ 0.37	62.64 $\pm$ 2.47	16.72 $\pm$ 0.66

\* PrFS Pre-flowering stage; PoFS Post-flowering stage



### *Impact of plantation time and phenological stages on the chemical composition of genotypes*

The data shown in Table II highlights the effect of planting time and phenological stages on the chemical composition ( $\alpha$ -cellulose, hemicellulose, lignin and extractive materials) of four different flax genotypes. Such variations can significantly influence the suitability of these genotypes for industrial applications, including textile, pulp and paper production, based on their fibre quality and chemical properties.

#### *BARI Tishi-1*

$\alpha$ -cellulose shows a progressive increase from 52.36% in the first planting to 61.62% in the third planting's post-flowering stage. This trend, paired with stable hemicellulose, suggests that later planting can enhance cellulose content, beneficial for applications requiring high-quality fibres. Lignin remains relatively stable across plantings, with a slight increase at the post-flowering stage in the third planting (7.65%). Extractives increase markedly at the post-flowering stage in the third planting (11.03%), suggesting higher accumulation of non-fibre substances like waxes, oils, and resins at later growth stages.

#### *BD-10708*

$\alpha$ -cellulose rises significantly from the first planting to the post-flowering stage of the third planting, reaching 64.34%, indicating that later stages might be more favorable for high-cellulose content, ideal for high-strength paper applications. Hemicellulose fluctuates but remains moderate, ensuring fibre flexibility without compromising cellulose concentration. Similarly, a notable increase in lignin (13.18%) occurs at the post-flowering stage of the third planting, which contrasts with a significantly low lignin content at the pre-flowering stage (3.43%). This genotype shows high extractives in the first planting (10.55%) but stabilizes across other plantings. This could imply that BD-10708 plants started early accumulate more extractive compounds initially, while later plantings are more chemically stable.

#### *Canada genotype*

The  $\alpha$ -cellulose content in Canada genotype remains relatively low and stable, with a slight increase to 50.13% at the post-flowering stage of the third planting. Hemicellulose peaks notably in the pre-flowering stage (34.23%), which may reduce fibre quality. The lignin content is highest in the

first planting (10.00%) then reduces and peaks again at the post-flowering stage of the third planting (11.34%). This indicates that early plantings and later stages might yield higher lignin, which could add structural rigidity but limit use in applications needing low lignin. Canada genotype exhibits consistently high extractive content, particularly at the pre-flowering stage in the third planting (10.48%), suggesting it may accumulate non-cellulosic substances.

#### *China genotype*

$\alpha$ -cellulose levels are the highest among all genotypes, increasing from 56.83% in the first planting to 62.64% at the post-flowering stage of the third planting. Hemicellulose content remains low and stable, making China genotype potentially the best candidate for high-quality cellulose applications due to its high and stable  $\alpha$ -cellulose content. This genotype's lignin content is relatively stable across stages and plantings, with a slight increase at post-flowering, maintaining a range of 8-9%. This stability indicates consistent fibre rigidity, beneficial for uniformity in end-use applications. Extractives vary moderately, with the highest in the pre-flowering stage of the third planting (8.41%) but lower values at the post-flowering stage (6.26%).

These findings highlight early plantings often exhibit higher extractive and lignin contents, while later plantings, especially at post-flowering stages, tend to enhance  $\alpha$ -cellulose levels.

### **Conclusion**

Each genotype is affected differently by the sowing date and growth stage, which both have a major impact on flax fibre yield and chemical composition, according to the study. With respect to fibre yields, the optimum sowing time of flax was 16 November. Among the genotypes, BARI Tishi-1 and China genotype show the highest  $\alpha$ -cellulose content, particularly in later plantings and post-flowering stages, making them ideal for applications requiring high cellulose content. BD-10708 also achieves high cellulose at later stages but with increased lignin, which may enhance rigidity for certain applications. The genotype Canada consistently shows high extractives and lignin. Overall, later plantings and post-flowering stages favour increased cellulose content. The study emphasizes the importance of considering sowing time and genotype selection in flax cultivation strategies to enhance fibre yield and quality.

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