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Relationship between the dynamic changes in polyamine pool and related parameters and nitrogen supply in maize plants under field conditions

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ABSTRACT

Polyamines are low-molecular-weight, amino group-containing compounds that have an essential role in plant growth and development. Although the relationship between polyamine metabolism and N assimilation, and, consequently, grain filling is becoming more obvious, the detailed metabolic connection is still unclear. Moreover, the dynamics of N supply-induced changes in polyamine metabolism have not yet been studied during maize plant development. This study demonstrated that changes in polyamine content and certain related parameters (nitrate and proline contents, the enzyme activities of diamine and polyamine oxidases) are N sensitive, and concentration-dependent tendencies were also observed. However, the N fertilisation induced well-characterised developmental stage-dependent changes in the polyamine and proline accumulation, and polyamine catabolism in both maize hybrids, differing in maturity and NUE potential. The metabolic shift during the vegetative to generative phase may be in relation to the grain filling-induced N remobilisation, for which polyamines are valuable sources. Changes in putrescine/spermidine and their relation with NO_3 content showed some temporal, genotype-dependent differences. The persistence of the correlation between putrescine and nitrate contents in the FAO 490 maize hybrid may suggest that the higher metabolic activity contributes to better N remobilisation and, in turn, higher yield upon NPK experiment. Based on the authors' findings, the developmental stage determines the extent of nutrient responsiveness, which underscores the importance of synchronising nutrient management with crop developmental stages. Therefore, monitoring of polyamine metabolism-related parameters can be an indicator of better N assimilation rate in the early reproductive stage until the R1 stage.

1. Introduction

Nitrogen (N), which makes up nearly 80% of the Earth's atmosphere, is an essential macroelement for living organisms, as a component of several organic compounds. Eukaryotic organisms cannot assimilate atmospheric N. Thus, the lack of useable N compounds is a common limiting factor for plant growth and development. However, extensive amounts of synthetic fertilisers are often required to ensure successful seed production and high grain yields in non-N-fixing food crops [1]. Given the projected increase in the world's human population, further increases in N input are expected, leading to many environmental problems. In general, only 30-50% of the N fertiliser is utilised by plants, while the remaining portion is partially utilised by soil microbes and a significant portion leaks into the groundwater, leading to the eutrophication of living waters [2]. The effort to reduce the environmental impact and improve the efficiency of N fertilisation is based on two distinct strategies. The first mitigation strategy focuses on application technology to reduce chemical N fertiliser use, by, for example, the application of slow-release fertilisers or bio-fertilisers, and proper crop management practices [3]. While the second target is the evaluation of plants with better N use efficiency (NUE) based on multiple performance indicators. As NUE of the cultivars can depend on the N input (form and dose), the identification of genotypes that exhibit a high, medium, or low N response is still an important and promising approach, for

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example, in wheat [4,5]. This approach requires well-defined characterisation at the phenotypic, metabolomic and genetic levels. Achieving greater NUE is challenged by the complexity of the trait, which comprises processes and pathways related to N uptake, transport, reduction, assimilation, translocation and remobilisation within plants [6]. Detailed research on defining phenotypic parameters for N-response in crops and revealing the genetic background is well-established [7–12]. Although metabolite profiling could be the missing link, showing the actual response of plants to N, and could be an accurate indicator of N assimilation [13], these kinds of analyses are still less common [14,15], where the PA metabolism was not the main focus.

Plants can assimilate different forms of N, but nitrate (NO_3^-) is the most abundant and generally preferred N source. Via reduction of NO_3, all N assimilated by plants ends up in three main amino acids, asparagine, glutamine (Gln) and glutamate (Glu). Glu as a metabolic branching point, is the precursor of several related metabolites like ornithine, arginine, proline and polyamines (PAs). PAs, including putrescine (PUT), spermidine (SPD), and spermine (SPM), are nitrogenous and polycationic compounds containing two or more amine groups. PAs are ubiquitously distributed in all cells at relatively high concentrations and regulate several processes during normal plant growth, development and stress responses at the cellular, physiological and biochemical levels (stabilisation of membranes and proteins, maintaining the osmotic balance, ROS scavenging, transcription, translation, photosynthesis, etc.) [2]. Their metabolism is fine-tuned by their biosynthesis, catabolism, and back-conversion (reviewed in Ref. [16]), and connected with other metabolic pathways (e.g., TCA cycle), which demonstrates their key role in the regulation of C and N metabolisms [17,18].

Although the primary N uptake- and assimilation-related genes have been well-studied [19,20], only a few studies focused on the effects of N supply on PA content [21–24]. Even though changes in PA metabolism are related to grain filling and N remobilisation, especially under abiotic stress conditions [25,26], the relation between the PA metabolism and N availability/uptake/assimilation is still not clear.

The main aims of the present work were 1) to evaluate how N or NPK fertilisation affects PA metabolism and certain other N metabolism-related parameters, namely PA levels, PA catabolism enzyme activities, proline and NO_3^- content across maize developmental stages, 2) to compare treatment responsiveness between two maize hybrids differing in maturity and NUE potential. It was hypothesised that N supply modifies the PA metabolism, which may be in relation to yield-related parameters. The authors hoped that the results might highlight which compounds are the most indicative elements of higher and balanced N assimilation or remobilisation efficiency, especially in the early period of plant growth.

2. Materials and methods

2.1. Plant material, growth conditions and treatments

To reveal the above-mentioned relations, the following experimental setup was conducted. Two different maize hybrids grown under field conditions were fertilised with different nutrient levels, and the investigated parameters were compared to each other at dedicated vegetative (V) and reproductive (R) stages.

Two maize (*Zea mays* L.) hybrids with different maturity groups were used in the research. The FAO 420 is a compact-type hybrid characterised by a strong stalk and a well-developed root system. It exhibits excellent early vigor and has consistently produced stable, high yields across multiple seasons in breeding trials. This hybrid is of the "stay-green" type and is recommended for sowing during the optimal maize planting window. The FAO 490 is a long-season, high-yield potential hybrid with outstanding drought tolerance. It also belongs to the stay-green type and has shown remarkable yield stability under variable weather conditions during a multi-year experiment.

The experimental sampling was part of an experimental design with a split-strip plot multifactorial long-term fertilisation experiment with four repetitions. The main factor was the fertilisation, creating the levels according to Table 1, with different N and NPK levels plus the sub-factor was the hybrid. The fertilisation levels were performed in four blocks, as physical replicates, with randomised position of the fertilisation levels within a block, to avoid the effects of soil heterogeneity on the fertilisation levels. The experiment was established by János Nagy in 1983 and has been conducted at the same location ever since, using identical agrotechnical parameters [27]. The soil characteristics of the experimental plot are summarised in Suppl. Table 1. Basal fertiliser application was carried out on October 6 (Table 1). Ploughing was performed on October 17th, followed by ploughing closure on February 22nd. N fertilisation according to the different N levels was applied on the plot level on April 9th, immediately followed by seedbed preparation. Sowing was carried out on April 11th, 2024 with 73.400 plant ha^{-1} plant density. Herbicide application followed on May 10th, using the tembotrione active ingredient containing herbicide (2 L ha^{-1}). Mechanical weed control was performed on May 16th using an inter-row cultivator. On September 23rd, the harvest was carried out using a Sampo Rosenlew SR2010 two-row plot-size combine harvester.

Leaf sample collection was performed in three replicates, at the same time of the day (9:00 a.m.) during each phenological stage (V6 – 6th leaf stage, V12 – 12th leaf stage, R1 – silking stage, R3 – milk stage, R6 – physiological maturity stage). In the V6 and V12 growth stages, samples were taken from the last fully developed leaf, whereas in the generative phase, leaf tissue opposite to the ear was collected. During sampling, leaf samples were collected from multiple plants per plot for each treatment and genotype. After removing the basal, apical, and midrib sections, the collected plant tissues were immediately placed in liquid nitrogen (LN_2). Samples were subsequently stored at −80 °C until analysis. Before biochemical analysis, the collected leaves were ground in LN_2. All chemical components were analysed from the same homogenate to ensure they originated from the same biological replicate. The procedure was performed in triplicate for each treatment-genotype combination, resulting in three biological replicates.

2.2. Agrometeorological parameters of the growing season

In the year 2024, January precipitation exceeded the long-term average by > 10 mm, whereas February - April were notably drier than average. In May, rainfall was 16 mm above the mean, coinciding with key vegetative stages of maize. June precipitation was near average, while July - August were markedly drier; combined with elevated temperatures. This tendency led to drought conditions from July onward. The pre-harvest period was warm and wet. Overall, early-season conditions were favourable for maize production, however, it was followed by summer precipitation deficits and heat stress.

2.3. Measurement of yield-related traits

Yield was measured when harvesting using the Shampo Rosenlew SR2010 plot-size harvester and its built-in scale to determine the yield/plot in kg ha^{-1}, then it was calculated to 1 ha using the exact plant density. 1 kg mean sample was taken from every harvested plot sample to measure the protein and moisture content, with a Perten DA7250 NIR analyser. Yield quantity was then equalised to 14% grain moisture

Table 1
The levels of the macronutrient used in the experiment as fertilizer supply.

(kg/ha)	N	P_2O_5	K_2O
N 0	0	0	0
NPK0	0	0	0
NPK60	60	46	54
N120	120	184	216
NPK120	120	92	108

content. Grain N concentration was calculated from this NIR-based protein content using a N-to-protein conversion factor of 6.25 for maize.

The grain N uptake (GNU) was calculated according to the following equation [28]:

$$\text{GNU}\ (\text{kg N ha}^{-1}) = \frac{\text{grain yield}\ (\text{kg } ha^{-1})\ \text{x grain N content}\ (\%)}{100}$$

2.4. Polyamine extraction and analysis

Each leaf sample was homogenised in liquid N_2, and 0.2 g was extracted in 2.0 ml solution of ice-cold 0.2 M $HClO_4$. After centrifugation for 10 min at 4 °C with 15 000 rpm, the supernatant was used for pre-column derivatisation with dansyl chloride, according to Németh et al. [29]. The following PA compounds were detected after being separated on a reverse phase Kinetex column (C18, 100 × 2.1 mm, 5 μm, Phenomenex, Torrance, CA, USA) by HPLC system (W2690 separation module and a W2475 scanning fluorescence detector, Waters, Milford, MA, USA): 1,3-diaminopropane (DAP), PUT, cadaverin (CAD), SPD, and SPM. Gradient elution was performed using mobile phase A (44% acetonitrile) and mobile phase B (acetonitrile: methanol = 7:3). The program started at 100% A, reaching 97 B over 13 min, then increased to 100% B over 1 min and was held for 4 min. Subsequently, the system was returned to 100% A for a 12-min equilibration period. The flow was 0.5 ml min $^{-1}$, and the column was thermostated at 40 °C. Excitation at 340 nm and emission at 515 nm were applied for detection.

2.5. Determination of nitrate content

Determination of nitrate content was carried out as described in Doneva et al. [30]. 0.2 g plant material was extracted with 1 ml ultrapure water and incubated at 80 °C for 60 min. After centrifugation (15 000 rpm, 10 min), the supernatant was filtered through a 0.45 μm pore size membrane filter. For the separation of nitrate, a Synergi 4 μm Fusion-RP 80 Å 150 × 4.6 mm column was used with isocratic elution using a mixture of 1-pentanesulfonic acid (1.74 g l^{-1}) and methanol (7:3) on a Waters HPLC system (Waters, Milford, MA, USA). Detection was carried out by a W996 UV/VIS photodiode array detector at 225 nm.

2.6. Determination of proline content

The quantification of proline (PRO) content was carried out according to the Bates method [31], which relies on its reaction with ninhydrin. In short, in the slightly modified method, 200 mg leaf samples were homogenised in 2 ml of distilled water. After centrifugation at 15000 rpm for 10 min at 4 °C, the supernatant was used for the reaction mixture, which consisted of 0.5 ml supernatant, 0.25 ml of glacial acetic acid and 0.25 ml of ninhydrin reagent. After incubating at 96 °C for 30 min, the chromophore generated was extracted using 1 ml of toluene. Its absorbance was determined using a UV-VIS Shimadzu 160A spectrophotometer (Shimadzu Corp. Kyoto, Japan) at 518 nm. The proline calibration curve was used for the quantification.

2.7. Measurement of diamine oxidase and PA oxidase enzyme activities

The activities of enzymes participating in PA catabolism, diamine- (DAOs, EC 1.4.3.6) and PA oxidases (PAOs, EC 1.5.3.3) were determined by a spectrophotometric method employed by Takács et al. [32]. Briefly, 0.2 g of leaf samples was homogenised in liquid N_2 and then in 0.6 ml extraction buffer containing 0.2 M Tris(hydroxymethyl)aminomethane (pH 8.0), 10% glycerol, 0.25% Triton X-100, 0.5 mM phenylmethanesulfonyl fluoride (PMSF); 0.01 mM leupeptin and 100 mM potassium phosphate buffer (pH 6.6). After centrifuging (10 min, 15 000 rpm, 4 °C) the supernatant was used. The reaction mixture contained 150 μl of supernatant, 600 μl 0.1 mM potassium phosphate buffer (pH 6.6), and 150 μl of 20 mM PUT for DAO or 150 μl of 20 mM SPM for PAO measurements. After incubation at 37 °C for 1.5 h, the reaction was stopped with 50 μl of 20% (w/v) trichloroacetic acid, which after 50 μl of 2-aminobenzaldehyde (0.1%) was also added. The formation of Δ^1-pyrroline was determined by measuring the absorbance at 430 nm (Shimadzu 160A). Enzyme activity was expressed in nmol Δ^1-pyrroline min^{-1} g^{-1} leaf fresh weight (FW) using an extinction coefficient of 1.86 x 103 mol^{-1} cm^{-1}.

2.8. Statistical analysis

The results of spectrophotometric and HPLC analyses represent at least three biological replicates. The data were visualised using the Microsoft Windows® Excel program. Duncan's post-hoc test was performed using SPSS 16.0. Significant differences were indicated with different letters ($p < 0.05$). A heat map with a dendrogram (Pearson correlation) for biochemical parameters was performed in OriginPro for the two maize hybrids, separately.

A factorial analysis of variance (ANOVA) was applied to evaluate the effects of phenological stage (P), genotype (G), and fertilisation level (F), including their interactions (P × G, P × F, G × F, and P × G × F), on the studied parameters, with a significance threshold of $p < 0.05$. When significant differences were detected, pairwise comparisons among treatment means were performed using Duncan's post-hoc test for biochemical traits and Tukey's honestly significant difference (HSD) test for yield-related traits. Pearson's correlation analysis was applied to quantify the strength and direction of linear relationships among the examined physiological parameters. Correlation coefficients (r) were calculated, and their statistical significance was assessed at a predefined probability level ($p < 0.05$). Statistical analyses for yield-related traits were performed in Minitab 22 statistical software.

3. Results

3.1. Grain yield and grain N content

Grain yield was significantly affected by the given maize hybrid and the applied nutrient treatment, with clear genotype-dependent yield responses. For the FAO 420 hybrid, yield under unfertilised control conditions (N0 and NPK0) was low, ranging from 6.29 to 7.34 t ha^{-1}, indicating limited yield potential in the absence of nutrient supply. The application of fertilisation resulted in a strong yield response: it increased to 10.55 t ha^{-1} at N120, and to 9.98 t ha^{-1} and 10.98 t ha^{-1} at NPK60 and NPK120, respectively. In the case of the FAO 490 hybrid, yields were consistently higher across all treatments. Even under unfertilised control conditions, yield ranged from 7.59 to 9.48 t ha^{-1}. Fertilisation markedly enhanced yield: 10.63 t ha^{-1} at N120, 12.68 t ha^{-1} at NPK60, and a maximum of 13.30 t ha^{-1} at NPK120, which formed the statistically highest yield group (Fig. 1.).

Grain N concentration was also influenced by nutrient level, while differences between hybrids were less pronounced, indicating a broadly similar N accumulation pattern in the two hybrids. For the FAO 420 hybrid, N starvation (N0 and NPK0) resulted in 0.95% and 0.93% grain N concentrations, respectively. Fertilisation significantly increased grain N content, with the statistically highest value at N120 (1.07%). Balanced fertilisation at NPK60 (1.05%) also maintained elevated grain N levels, while a further increase in nutrient supply (NPK120) did not result in additional N accumulation in the grain (0.99%), suggesting a dilution effect associated with higher yield formation. A similar trend was observed for the FAO 490 hybrid. Grain N concentration without N supply ranged from 0.90 to 0.96%, with the lowest value under NPK0. While fertilisation markedly increased grain N content, reaching the statistically highest category (1.08%) again at N120. Balanced treatments, NPK60 (1.03%) and NPK120 (1.04%), maintained higher grain N levels than the control but did not exceed those achieved at N120. Overall, the obtained results suggest that higher N supply alone (N120) was sufficient to maximize grain N concentration in both hybrids.

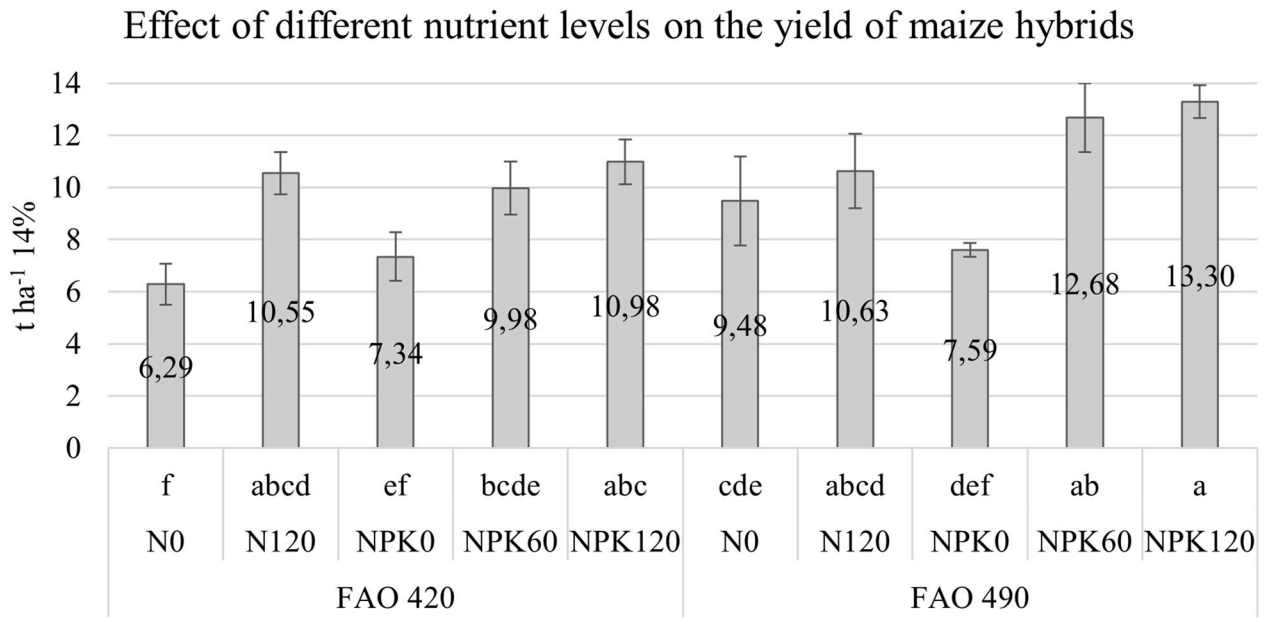

Fig. 1. Effect of different nutrient levels on the yield of maize hybrids (FAO 420 and FAO 490). Yield: t ha^{-1} at 14%. n = 4 ± SE. Means marked with different letters are statistically different.

Higher nutrient levels did not further increase grain N content in the NPK treatments, likely due to increased carbohydrate deposition and yield-driven dilution effects (Fig. 2.). Results of the analysis of variance of yield and grain N content between factors "Genotype", and "Fertilisation level" has been presented in Suppl. Table 2.

High calculated GNU values were reached for FAO 420 hybrid after both N120 and NPK120 fertilisations, 100.2 and 104.3 kg N ha^{-1} (with a grain yield level of 10.55 and 10.98 t ha^{-1}, respectively) (Table 2). In the case of FAO 490 hybrid, NKP60 and NPK120 treatments resulted in the highest GNU values of 120.5 and 126.4 kg N ha^{-1}, which means 167 and 175% increases, respectively (Table 2). These values further confirmed the differences in the NUE of the two investigated hybrids. The authors' findings suggest that grain quality, in terms of N (protein) concentration, can be optimised with only N inputs independently of maximum yield levels in FAO 420, but not in FAO 490.

3.2. Determination of PA metabolism-related parameters

The initial levels of the investigated parameters (DAP, PUT, CAD, SPD, SPM and PRO contents, and DAO and PAO enzyme activities) were quite similar in the two hybrids and changed similarly during the experiment due to the senescence. The most remarkable changes observed after the transition from the V to R phase were the decrease of DAP, PUT, SPD levels, but the increase in PAO activity in both hybrids (Tables 3 and 4).

Increasing tendencies were detected in DAP, PUT, SPD, SPM and PRO contents after N or NPK fertilisation at all the sampling times (V6, V12, R1, R3 and R6), in both hybrids. However, more significant

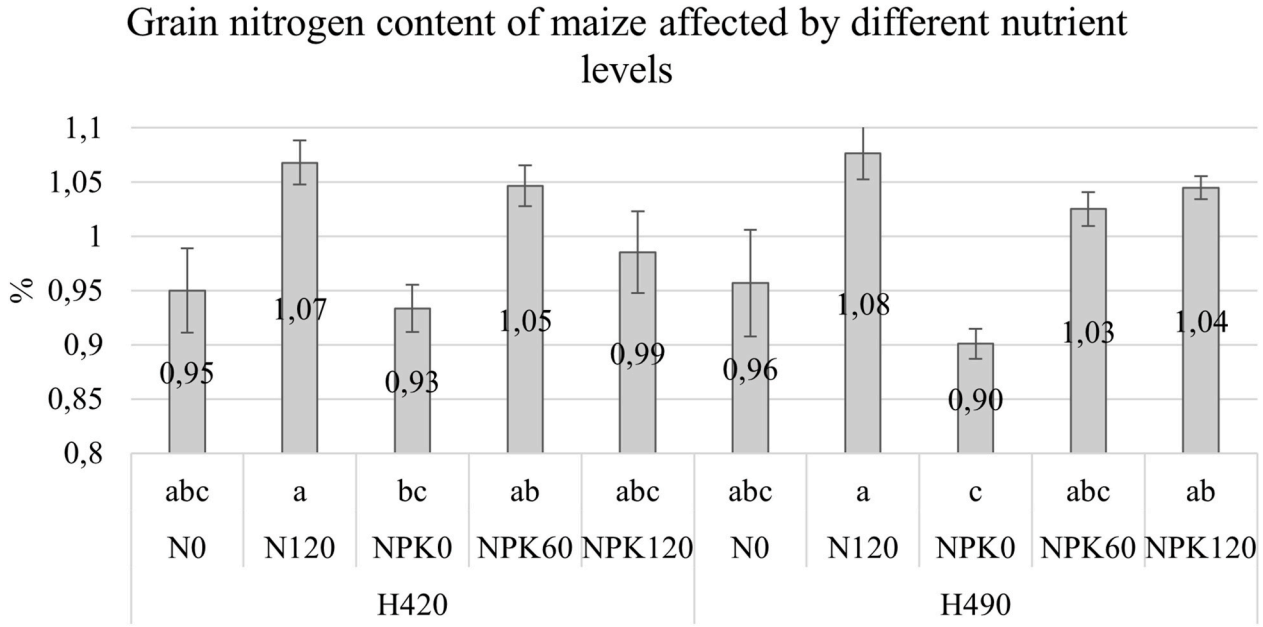

Fig. 2. Grain nitrogen content (%) of maize affected by different nutrient levels. n = 4 ± SE Means marked with different letters are statistically different.

Table 2
Grain nitrogen uptake (GNU).

	FAO 420					FAO 490				
	N0	N120	NPK0	NPK60	NPK120	N0	N120	NPK0	NPK60	NPK120
GNU kg N/ha	59.5	100.2	69.7	94.8	104.3	90.1	101.0	72.1	120.5	126.4

Table 3
Changes in cadaverin (CAD), 1,3 diaminopropane (DAP), nitrate (NO_3), proline (PRO), putrescine (PUT), spermidine (SPD) and spermine (SPM) contents, and the enzyme activities of diamine oxidase (DAO), polyamine oxidase (PAO) during the experiment in 420 maize genotype. Significant differences were indicated with different letters ($p < 0.05$).

		DAP	PUT	CAD	SPD	SPM	PRO	DAO	PAO	NO_3
V6	**N0**	3.6 ± 0.8 de	22.8 ± 1 c	0.04 ± 0.00 b	29.3 ± 3.3 c	8.1 ± 0.7 defg	12.5 ± 1.4 bcd	27 ± 1.4 def	12.2 ± 2.4 ijk	234 ± 29 efg
	N120	5 ± 1.1 cd	21.4 ± 3.3 cd	0.11 ± 0.11 b	33.4 ± 3.6 bc	10.8 ± 1.6 bcde	15.8 ± 0.9 a	24.5 ± 1.2 efgh	8.7 ± 4.1 k	644 ± 91 a
	NPK0	3.9 ± 0.9 cde	43.6 ± 6.8 a	0.19 ± 0.25 b	39 ± 5.4 b	6.8 ± 2.4 efg	14.7 ± 1.4 ab	26.1 ± 2.9 efg	13 ± 6.1 hijk	261 ± 48 efg
	NPK60	4.8 ± 0.7 cd	39.9 ± 4.9 a	0.19 ± 0.11 b	47.1 ± 5.9 a	9.3 ± 0.4 bcdef	16.6 ± 0.9 a	22.2 ± 0.7 fghij	10.2 ± 4.6 jk	419 ± 34 b
	NPK120	4.9 ± 1 cd	26.1 ± 2.8 bc	0.16 ± 0.00 b	33.9 ± 5.4 bc	7.4 ± 3 efg	14.8 ± 1.9 ab	20.2 ± 3.6 ghijk	6 ± 2.1k	746 ± 151 a
V12	**N0**	5.2 ± 0.7 cd	9.1 ± 1.3 ghij	0.29 ± 0.37 b	21.7 ± 6.5 de	5 ± 2.1 g	9 ± 1.6 fghi	22.9 ± 5 efghi	21.5 ± 10.1 ghij	294 ± 23 efg
	N120	9.8 ± 1.6 a	27.3 ± 2.8 b	0.05 ± 0.04 b	35.1 ± 4.2 bc	8.8 ± 2.2 cdefg	14.4 ± 1.8 abc	16.7 ± 1.2 ijkl	21.5 ± 3.2 ghij	439 ± 76 bcd
	NPK0	5.5 ± 1.3 c	10.8 ± 2.2 ghij	0.16 ± 0.01 b	22.8 ± 3 de	4.9 ± 0.7 g	8 ± 1.1 ghij	18.9 ± 2 hijkl	23 ± 3.3 ghi	300 ± 11 efg
	NPK60	9.5 ± 2.4 ab	22.4 ± 4.1 cd	0.04 ± 0.00 b	31.5 ± 9.5 c	10.3 ± 6.9 bcdef	8.9 ± 0.5 fghi	13.1 ± 1 l	25.8 ± 2.3 g	434 ± 82 bcd
	NPK120	8.1 ± 1.1 b	28.9 ± 1.7 b	0.05 ± 0.01 b	35.3 ± 2.3 bc	10.1 ± 0.4 bcdef	10.5 ± 1.4 defg	16.1 ± 2 jkl	13 ± 2.8 hijk	462 ± 84 bc
R1	**N0**	1.2 ± 0.4 fghi	4.1 ± 0.1 jkl	0.27 ± 0.04 b	13.2 ± 0.7 fg	10 ± 1.8 bcdef	8 ± 0.5 ghij	33.3 ± 4.5 bcd	37.1 ± 4.5 f	183 ± 22 g
	N120	5.5 ± 2.2 c	17.9 ± 4.2 de	0.57 ± 0.71 ab	19.4 ± 1.9 def	16.4 ± 0.7 a	12.8 ± 1.4 bcd	18.6 ± 1.6 cdef	22.8 ± 2.3 ghi	297 ± 75 efg
	NPK0	1.2 ± 0.2 fghi	5.3 ± 1.3 jkl	0.27 ± 0.1 b	13.7 ± 2.3 fg	10 ± 2.1 bcdef	11.2 ± 2.5 def	32.6 ± 5.3 bcd	25.6 ± 5.4 g	261 ± 30 efg
	NPK60	1.2 ± 0.1 fghi	5.2 ± 0.9 jkl	0.18 ± 0.05 b	15 ± 1.1 efg	10.7 ± 0.7 bcde	12 ± 2.4 cd	28.3 ± 2.1 cdef	23.9 ± 4.3 gh	247 ± 26 efg
	NPK120	2.8 ± 0.6 ef	11.5 ± 0.7 fgh	0.34 ± 0.38 b	17.1 ± 1.2 defg	13.2 ± 0.4 ab	11.8 ± 2.6 cde	22.2 ± 2.3 fghij	16.7 ± 0.6 ghijk	246 ± 58 efg
R3	**N0**	0.6 ± 0.2 ghi	4.1 ± 1.3 jkl	0.52 ± 0.63 ab	11.4 ± 1.4 f	6.8 ± 1 efg	6 ± 0.1 kl	25.2 ± 2.7 efg	60.1 ± 4 ab	237 ± 24 efg
	N120	2.3 ± 0.2 efg	13.2 ± 1.2 fg	0.04 ± 0.00 b	13.3 ± 3 fg	8.3 ± 2.5 defg	11 ± 1.6 def	16.7 ± 2.4 ijkl	37 ± 19.9 f	262 ± 12 efg
	NPK0	0.7 ± 0.2 ghi	5.7 ± 2 jkl	0.57 ± 0.42 ab	12.6 ± 0.2 fg	6 ± 0.9 fg	5.3 ± 0.4 l	28.6 ± 7.4 cdef	53.2 ± 2.3 abcd	214 ± 7 fg
	NPK60	1.5 ± 0.6 fghi	9 ± 2.8 ghij	0.09 ± 0.07 b	15.2 ± 1.3 efg	10.2 ± 1.8 bcdef	7.8 ± 0.3 hijk	16.8 ± 2.3 ijkl	45.6 ± 2.4 def	185 ± 25 g
	NPK120	2.3 ± 0.3 efgh	14.9 ± 2.5 ef	0.48 ± 0.36 ab	16.8 ± 1.6 defg	9.6 ± 0.7 bcdef	10.6 ± 1.1 defg	14.3 ± 1 kl	39.4 ± 0.8 ef	199 ± 36 fg
R6	**N0**	0.5 ± 0.1 hi	2.6 ± 1 l	2.22 ± 3.22 ab	15.4 ± 1 efg	9.4 ± 2.7 bcdef	6.5 ± 0.6 ijk	46.5 ± 6.6 a	47.4 ± 6.3 cdef	328 ± 56 def
	N120	2.4 ± 0.5 efg	6.6 ± 1.9 ijkl	0.48 ± 0.03 ab	18.3 ± 2.5 defg	12.2 ± 1.6 bcd	12.3 ± 1.2 bcd	28.9 ± 3.2 cde	50 ± 9.1 bcde	297 ± 60 efg
	NPK0	0.3 ± 0.1 i	2.7 ± 0.9 l	2.56 ± 3.51 a	16.4 ± 5.3 defg	10.6 ± 0.9 bcde	6.5 ± 0.3 ijk	35.4 ± 4.3 b	50.9 ± 4.6 bcd	348 ± 35 cde
	NPK60	0.9 ± 0.5 ghi	3.8 ± 1.1 kl	1.52 ± 0.78 ab	16.1 ± 2.8 defg	12.8 ± 3.1 abc	9.4 ± 0.5 efgh	33.6 ± 0.8 bc	57.4 ± 7.2 abc	297 ± 49 efg
	NPK120	1 ± 0.2 fghi	7.9 ± 0.6 hijk	1.57 ± 2.17 ab	21.3 ± 0.7 de	13.2 ± 1.8 ab	13 ± 1.8 bcd	28.1 ± 3.2 cdef	62.8 ± 4.2 a	310 ± 112 efg

changes were found in the case of the FAO 420 hybrid than the FAO 490 one (Tables 3 and 4). In addition, in the FAO 420 hybrid, N or NPK fertilisation significantly increased the contents of PUT and PRO at all the sampling times (Table 3), with the most pronounced, and in most cases, concentration-dependent changes at V12. Here, especially PUT, SPD, SPM and PRO accumulation were induced by the fertilisation (N or NPK treatments). Interestingly, the NPK120 fertilisation resulted in similar values as found after N120 treatment in both hybrids, suggesting that the changes are the outcomes and dominant effect of the N supply, and the availability of P and K did not further influence them (Tables 3 and 4). Namely, the PUT content increased by 300 and 268% compared to the adequate control after N120 and NPK120 treatments, respectively. Similarly, the N120 and NPK120 fertilisation treatments increased the SPD content by 162 and 155%, respectively, and SPM content by 176 and 206%, while PRO showed increases of 160 and 131%, respectively, compared to the respective controls.

In the young leaves, the level of the catabolite product, DAP, was higher together with the amounts of PAs, and was tendentially N-inducible. While the enzyme activities of DAO and PAO were relatively low and did not change with the N supply. In contrast, in the R phase, DAP levels were very low, so the N supply could not influence them significantly. While the DAO and especially the PAO activities were

Table 4
Changes in cadaverin (CAD), 1,3 diaminopropane (DAP), nitrate (NO3), proline (PRO), putrescine (PUT), spermidine (SPD) and spermine (SPM) contents, and the enzyme activities of diamine oxidase (DAO), polyamine oxidase (PAO) during the experiment in 490maize genotype. Significant differences were indicated with different letters ($p < 0.05$).

		DAP	PUT	CAD	SPD	SPM	PRO	DAO	PAO	NO3
V6	**N0**	5.7 ± 1.7 bc	37.4 ± 12.2 b	0.29 ± 0.42 b	38.4 ± 14.4 abcd	7.9 ± 2.3 efgh	15.8 ± 2.9 abc	24.3 ± 2.1 hijk	10.9 ± 1.4 jk	303 ± 71 def
	N120	4.2 ± 0.2 c	27.9 ± 2.9 cd	0.11 ± 0.11 b	47.7 ± 9.9 ab	14.7 ± 4 abc	17.1 ± 1.7 ab	19.5 ± 4 jklmno	6.8 ± 2.1 k	601 ± 66 a
	NPK0	4.5 ± 1 c	50.9 ± 12 a	0.10 ± 0.09 b	39.9± 7.1 abcd	8.1 ± 1.2 efgh	14.1 ± 1.2 bcde	23.6 ± 2.4 hijkl	10 ± 1.7 jk	243 ± 74 ef
	NPK60	7.8 ± 3.5 a	54.1 ± 10.6 a	0.09 ± 0.05 b	48.5 ± 9.9 a	11.3 ± 3.2 cdef	18.2 ± 4.3 a	21.1 ± 1.6 ijklmn	13.6 ± 5.9 hijk	423 ± 91 bcd
	NPK120	7.5 ± 1.2 ab	33.4 ± 3.1 bc	0.04 ± 0.02 b	42.4 ± 3 abc	9.9 ± 2.5 defg	17.5 ± 3.2 ab	20 ± 2.5 jklmn	12.2 ± 4 ijk	812 ± 9 a
V12	**N0**	5.3 ± 1.2 c	11.7 ± 1.5 fghi	0.06 ± 0.03 b	26.2 ± 2.9 efg	6.6 ± 1 fgh	9 ± 1 fghij	20.8 ± 2.2 ijklmn	24.2 ± 6.2 fgh	267 ± 30 ef
	N120	8.8 ± 2.8 a	23.3 ± 4.3 de	0.03 ± 0.02 b	30 ± 13.3 def	6.7 ± 4.4 fgh	10.7 ± 1 efghi	15.4 ± 1.1 no	20 ± 5.2 fghij	337 ± 86 cdef
	NPK0	4.5 ± 0.3 c	14.5 ± 0.7 fgh	0.01 ± 0.00 b	32.7 ± 1.4 cde	9.2 ± 1.1 efgh	8.9 ± 0.8 fghij	18.9 ± 1.3 klmno	14.9 ± 4.8 hijk	462 ± 13 bc
	NPK60	8.4 ± 1.7 a	17.5 ± 1.4 ef	0.05 ± 0.00 b	27.6 ± 6.5 efg	6.7 ± 2.8 fgh	9.5 ± 1.2 fghij	15 ± 3.1 no	22.5 ± 1.6 fghi	507 ± 23 b
	NPK120	8.2 ± 0.5 a	23.2 ± 2.3 de	0.12 ± 0.07 b	37.7 ± 6.2 bcd	10.3 ± 3.2 cdefg	11.4 ± 1.5 defgh	13.8 ± 1.6 o	17.9 ± 2 ghijk	365 ± 33 cde
R1	**N0**	1.1 ± 0.3 e	7.2 ± 1.2 hijk	1.01 ± 1.03 bcd	18.1 ± 1.5 gh	12.3 ± 2.6 bcde	10.1 ± 2.2 fghij	33.4 ± 3.5 defg	29.1 ± 0.3 f	214 ± 39 ef
	N120	3.6 ± 0.8 cd	16.2 ± 2.5 efg	0.93 ± 1.28 bcd	20.8 ± 3.4 fgh	16.9 ± 3.1 a	14.5 ± 1 bcd	21.6 ± 1.4 ijklm	16.4 ± 3.4 hijk	329 ± 21 cdef
	NPK0	1.8 ± 0.1 e	4.7 ± 0.5 ijk	0.09 ± 0.01 d	13.2 ± 1 h	9 ± 0.9 efgh	8 ± 0.2 hij	33.7 ± 7.1 cd	28.3 ± 9.1 fg	228 ± 15 ef
	NPK60	0.5 ± 0.4 e	7.3 ± 3.8 hijk	1.32 ± 1.08 cd	17.9 ± 2.9 gh	14.4 ± 4.9 abcd	10.1 ± 4 fghij	25.1 ± 5 ghij	27.4 ± 5.4 fg	240 ± 60 ef
	NPK120	2 ± 0.5 de	7.7 ± 1.5 hijk	0.36 ± 0.42 bcd	17.1 ± 0.4 gh	16.5 ± 1.8 ab	9.4 ± 1.6 fghij	22.1 ± 2.5 hijkl	16.1 ± 3.5 hijk	299 ± 65 def
R3	**N0**	0.2 ± 0.1 e	5.8 ± 1.8 hijk	0.77 ± 1.21 cd	14.6 ± 2.5 h	5.4 ± 0.7 gh	6.3 ± 1.5 j	31.6 ± 4.5 def	55 ± 12.6 abc	212 ± 45 ef
	N120	0.7 ± 0.4 de	11.4 ± 1.3 fghij	0.89 ± 0.72 bcd	17.7 ± 2 gh	10.2 ± 2.5 cdefg	9 ± 1.2 fghij	15.9 ± 0.7 mno	48.1 ± 4.5 cd	204 ± 17 f
	NPK0	1.2 ± 0.1 e	5.3 ± 1.2 ijk	0.56 ± 0.37 cd	12.3 ± 2.2 h	4.8 ± 0.4 h	6.7 ± 0.3 j	38.9 ± 1.1 bc	62.2 ± 2.2 a	216 ± 17 ef
	NPK60	0.4 ± 0.4 e	6 ± 0.6 hijk	0.14 ± 0.07 d	12.9 ± 2.3 h	7.3 ± 2.5 fgh	7.5 ± 0.9 hij	22.2 ± 3.1 hijkl	49.9 ± 7.6 bc	208 ± 24 ef
	NPK120	1.3 ± 0.1 e	9.2 ± 2.4 fghijk	0.45 ± 0.68 cd	14.9 ± 1.7 h	8.7 ± 0.4 efgh	9.7 ± 0.7 fghij	17.9 ± 0.1 lmno	30.8 ± 2.3 ef	214 ± 8 ef
R6	**N0**	0.4 ± 0.4 e	2.4 ± 0.8 k	3.34 ± 2.47 ab	14.7 ± 2.5 h	6.9 ± 1.4 fgh	7 ± 1.2 ij	45 ± 6.9 a	53 ± 7.9 abc	330 ± 39 cdef
	N120	1.3 ± 0.2 e	9.3 ± 2.9 fghijk	1.47 ± 2.02 bcd	21.9 ± 4.9 fgh	9.6 ± 2.2 defgh	12.3 ± 1.2 cdef	27.7 ± 2.5 efgh	39.5 ± 7.1 de	243 ± 38 ef
	NPK0	0.3 ± 0.1 e	2.8 ± 0.8 jk	5.08 ± 3.27 a	14.9 ± 1.6 h	8.3 ± 1.9 efgh	8.5 ± 1 ghij	42.4 ± 4.2 ab	59.8 ± 4.4 ab	316 ± 23 cdef
	NPK60	0.4 ± 0.1 e	6.5 ± 2.3 hijk	2.90 ± 2.75 abc	17.8 ± 1.2 gh	9.7 ± 1 defg	8.9 ± 1 fghij	32.9 ± 1.1 de	57.4 ± 5.9 abc	289 ± 56 def
	NPK120	0.9 ± 0.2 e	8.9 ± 0.5 fghijk	2.61 ± 3.38 bcd	22.6 ± 1.3 efgh	12.6 ± 2 abcde	12.2 ± 3.5 defg	26.9 ±1.7 fghi	51.4 ± 11.3 bc	277 ± 40 def

higher than in the V6 and V12 phases, and N treatments could decrease them (Tables 3 and 4). In the FAO 420 hybrid at the V12 stage, the PUT content significantly increased after N supply together with the free NO_3 content (149, 144 and 154% increases after N120, NPK60, and NPK120 treatments, respectively). This correlation disappeared at the R1 and R3 stages; furthermore, at the R6 stage, N treatment increased the PUT level, but decreased the NO_3 content. Compared to these, in the FAO 490 hybrid, the positive correlation between PUT and NO_3 contents still persisted at the R1 phase (with 153, 105 and 131% increases after N120, NPK60 and NPK120 treatments, respectively), while it disappeared at the R3 stage, and turned also into a negative relation at the R5 stage.

Although the results presented in Tables 3 and 4 were largely similar; certain slight differences were also revealed due to a hierarchically clustered heatmap (Fig. 3A and B). In the FAO 420 hybrid, the correlation was high between the levels of DAO/PAO and SPM, and these were separated from the group of PUT-SPD-PRO-DAP-NO_3. While in the FAO 490, the SPM was clustered rather closer to this latter group, far from DAO and PAO. Regarding the performed treatments, in both hybrids, the treatments were clustered according to the V or R phases. In the case of the FAO 420 hybrid, both at the V6 and V12 phases, the treatments were in close correlation according to the N starvation or supply, namely, the N0 and NPK0 treatments, and the N120 and NPK60 or NPK120 were clustered separately. It was true in the case of the FAO 490 hybrid only at the V12 phase. However, it should also be mentioned that at later developmental stages, N120 and NPK120, or N0 and NPK120 treatments are often clustered together.

As the obtained results showed that NPK120 and N120 treatments had similar effects in both hybrids, Pearson's correlation analysis was performed only on the results of NPK treatments. The analysis confirmed close relationships between the investigated parameters (Fig. 4.). Close, positive correlations were found between PUT and SPD, PUT or SPD and PRO, PUT or SPD and DAP, DAO and PAO, while negative correlations were found between DAO or PAO and DAP, in addition between PUT or SPD and PAO. The results of the variance analysis (involving the results of both hybrids from the NPK treatments) clearly demonstrated that the phenological stage was the most influential factor across all measured parameters (Suppl. Table 3.). It had a highly significant effect on DAP, PUT, CAD, SPD, SPM, PRO, DAO, PAO, and NO_3 (all $p < 0.001$), confirming the dominant role of developmental timing in shaping physiological and biochemical responses. This consistent influence highlights

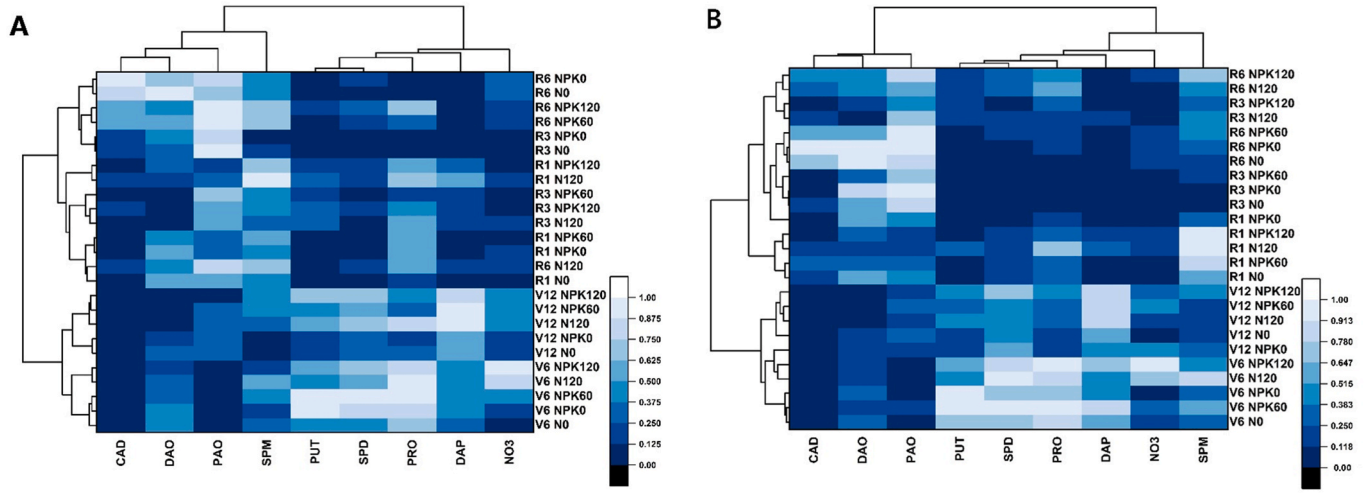

Fig. 3. Heat map with dendrogram of the normalised data of cadaverin (CAD), 1,3-diaminopropane (DAP), nitrate (NO_3), proline (PRO), putrescine (PUT), spermidine (SPD) and spermine (SPM) contents, and the enzyme activities of diamine oxidase (DAO), polyamine oxidase (PAO). Results of the FAO 420 hybrid were shown on panel A, while those of the FAO 490 hybrid were shown on panel B.

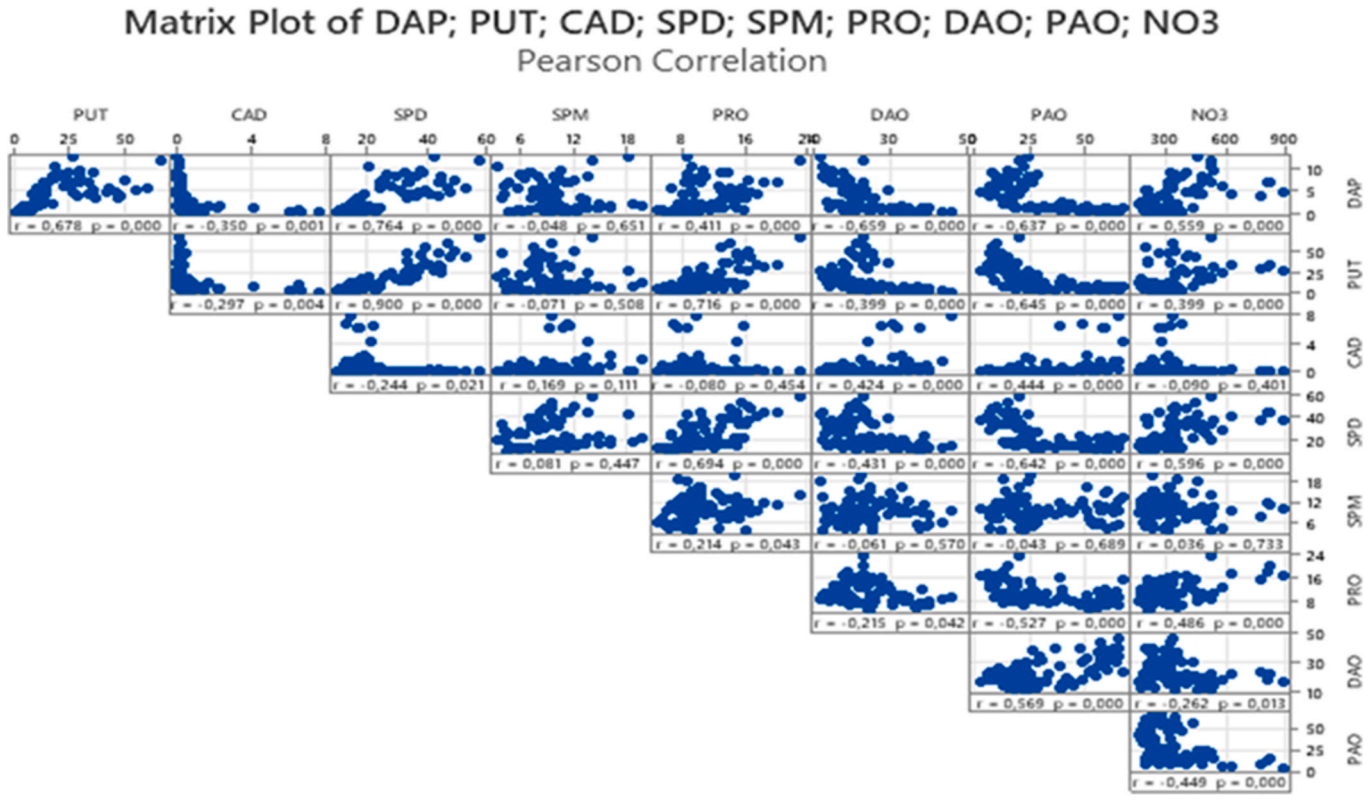

Fig. 4. Pearson correlation on the data obtained on both hydrides during the NPK treatments at all sampling times. Cadaverin (CAD), 1,3-diaminopropane (DAP), nitrate (NO_3), proline (PRO), putrescine (PUT), spermidine (SPD) and spermine (SPM) contents, and the enzyme activities of diamine oxidase (DAO), polyamine oxidase (PAO).

that changes in plant growth stage were the primary driver of variation in both growth-related and metabolic traits. Fertilisation level also played an important role, though its effect was parameter-dependent. It significantly influenced PUT, SPD, SPM, PRO, DAO, PAO, and NO_3 (all $p < 0.001$), with particularly strong effects on DAO, PAO, and NO_3. In contrast, fertilisation level had no impact on CAD. These results emphasise that fertilisation affects both accumulation processes and metabolite dynamics, but not all traits are equally sensitive to nutrient input. Genotype effects were generally weak; namely, significant differences were observed for PUT ($p = 0.015$) and SPD ($p = 0.004$), while DAO, PAO, and NO_3 were not affected. This suggests that genetic background contributed only marginally compared with developmental and fertilisation effects in these investigated hybrids. Interaction effects revealed additional complexity (Suppl. Table 3.). The combination of phenological stage x fertilisation level was the most consistently significant, influencing PUT, SPD, PRO, DAO, PAO, and NO_3 (all $p < 0.05$). This interaction highlights that fertilisation effects were strongly growth stage-dependent, with developmental timing determining the extent of

nutrient responsiveness. In contrast, phenological stage x genotype was significant only for DAP, PUT, SPM, and DAO, indicating that genotypic differences became evident at particular developmental stages but did not persist across all traits. The three-way interaction (phenological stage x genotype x fertilisation level) was significant only for PAO ($p = 0.008$).

4. Discussion

The plant N content is determined by the uptake of inorganic N and then its assimilation, as well as the transport, metabolism, distribution and storage of N-containing compounds [33]. During the grain filling in cereal crops, the N content significantly increases along with protein synthesis that determines the grain quality. However, a significant portion of grain N does not originate from freshly taken up soil N, but rather from the remobilisation from the leaves [34]. Improving plant NUE also requires clear knowledge of N remobilisation, in addition to the understanding of N uptake and assimilation of a given plant species [6]. The highlighting and revealing of the metabolic relationship between the primary products of N assimilation (Glu, Gln, or asparagine) and other N-containing compounds, such as PAs or proline, is also necessary.

PAs are amino group-containing, low-molecular-weight compounds that are present in all living cells. They have an essential role in plant growth, development, stress tolerance, flowering and yield production [35–37]. As they are also sinks of the assimilated N, PA metabolism is sensitive to the availability of N during the vegetative phase. N supply can easily and promptly modify the level of PAs and some PA metabolism-related parameters [38]. After the transition from V to R stage, during the grain filling, N remobilisation begins with leaf senescence. PAs have a dual role in this period, partly due to their antioxidant roles; they can delay senescence [39], allowing the plant enough time to "empty" organic N from older leaves. Characterisation of barley PA transporters suggested that the upregulation of them has a role in N remobilisation, which was also supported by the fact that their expression decreased and was the lowest at the last stage of the grain filling [40]. According to these factors, PA synthesis may act as a kind of "buffer". It became clearer that the PA-N interplay in plants is of major interest because it connects N and C assimilation, and secondary metabolism pathways [41]. Certain studies revealed that the grain filling rate and/or weight, or grain N content were positively correlated with SPD and SPM accumulation, especially under stress conditions or after PA treatments in wheat or barley [25,42–45]. However, the dynamics of N-PA relationship have not yet been studied during maize plant development from the early V to the R phases. Maize, as a C4 plant with relatively high NUE, can be deployed as a good candidate for understanding better N uptake [8]. In the present study, the authors aimed to monitor the effects of N supply (either alone or as NPK) at different concentrations on PA content and on across maize developmental stages to compare the treatment responsiveness of two maize hybrids differing in maturity and NUE potential.

In the present experiment, grain yield and grain N concentration responded differently to N and NPK fertilisation, with clear hybrid-specific patterns. The FAO 420 hybrid showed a strong response to N supply, but only a limited additional benefit was observed after NPK fertilisation. These results suggest that this hybrid responds positively to fertilisation, although yield gains tend to plateau beyond moderate nutrient levels. In contrast, in the case of the FAO 490 hybrid, yields were consistently higher across all treatments, demonstrating superior baseline performance and greater tolerance to nutrient limitation, in addition responded strongly to balanced NPK supply. Across both hybrids, N120 was sufficient to maximize grain N content, NPK treatments did not further increase it, and in the case of the FAO 420 hybrid, they even slightly reduced it, indicating a yield dilution effect.

To obtain a more detailed insight into the dynamics of N assimilation and its relation with PA metabolism, in addition to the level of the most abundant PAs, namely the diamine PUT, the triamine SPD and the tetraamine SPM, other PA metabolism-related parameters were also detected. The enzyme activities of apoplastic DAO and PAO - the former is responsible for the terminal oxidation of diamines, and the latter that of the higher PAs (SPD and SPM)- were also measured to get information about the intensity of the terminal catabolism of PAs. The amount of the catabolite product of higher PAs, DAP, was also determined. The level of CAD, which is also a diamine (like PUT), but synthetised differently from the PUT-SPD-SPM pathway, was also detected. PRO a multifunctional amino acid that functions both as a stress marker and a protective and signalling compound, and even an energy source in plants [46,47]. The synthesis of PRO and PUT partly overlaps, as beginning with the same precursor, Glu; in addition, the catabolism of PAs may also be implicated in PRO synthesis. Accordingly, monitoring the level of PRO was also an objective. The detection of free NO_3 content may also provide further information on the actual N metabolism of plants.

The initial levels (at the V6 stage) of the investigated PA metabolism-related compounds were quite similar, and senescence induced similar tendencies in both genotypes during the experiment. Statistical analyses confirmed that the phenological stage was the most influential factor across all measured parameters. The levels of PAs (PUT and SPD) and even that of PRO were higher in the younger leaves compared to the R phases. These changes are consistent with the fact that in the young plants, the higher level of PAs is essential for intensive plant growth and development, but later on, during senescence (grain filling), catabolic processes are dominant [48]. In the young leaves, together with the amounts of PAs, the level of the catabolite product, DAP, was also higher, and was tendentially inducible by the N fertilisation, which suggests that N supply effectively activates PA metabolism. While the enzyme activities of DAO and PAO were relatively low and did not change with the N supply, these further support the hypothesis that the fine-tuned balance of PA synthesis and catabolism is important in plant growth and development. Nevertheless, the pronounced decrease of PUT content during the transition of V to R stage can reflect on the increasing remobilisation and translocation of the leaf N stored in PAs to the grains. DAP levels were very low, so the N supply could not significantly influence them. While the DAO and especially the PAO activities were higher than in the V6 and V12 phases, and N treatments could decrease them, maybe in order to compensate for the decrease of the PA pool under a critical level.

Usually, a negative correlation can be detected in plants between the leaf ontogenic stage and the PA levels; and it was also demonstrated that PAO-mediated catabolism of SPD/SPM is involved in, for example, dark-induced senescence [16,49]. Despite these increasing and decreasing tendencies during the experiment, N supply could induce specific changes in almost every phenological stage. Dominant increasing effect of N120 and NPK120 on PUT, SPD, SPM, and DAP, in addition to PRO, was more pronounced from the V12 stage. N supply increased the NO_3 content at the V6 stage, but at the V12 stage, this was not pronounced, and from the R1 stage it was not significant. Although it has been reported that NO_3^- supply reduced the total PA level in response to NH_4^+ stress in wheat [24], other studies reported a positive correlation between NO_3^- fertilisation and PA accumulation. Different anionic forms of N (KNO_2, KNO_3, $NaNO_2$ or $NaNO_3$) also resulted in prolonged PA accumulation in wheat [50]. The PA content, in addition to the activities of PA-synthesising enzymes, was increased at a higher N dose compared to the recommended dose, and a sub-optimal N dose induced PA catabolising enzymes (DAO and PAO), resulting in higher PA oxidation levels to cope with N deficiency in wheat [38]. Interestingly, in the present experiment, N120 and NPK120 treatments decreased the activities of DAO and PAO enzymes, indicating that sufficient N application is favourable for anabolic processes and N storage in PAs [51]. Similarly, in tomato, copper amine oxidases genes were found to be differentially expressed in response to various N fertilisation doses, indicating modulation of PUT availability in response to N supply [52].

Concentration-dependent differences were also found during NPK60

and NPK120 application compared to the NPK0 treatment, especially in the case of PUT, SPM, DAP, DAO and PAO, but not at all phenological stages. Nevertheless, under the present conditions, the NPK120 and N120 treatments had similar effects on the investigated parameters in both hybrids. It is known that the application of balanced N fertilisers may promote a wider range of metabolic processes and all major aspects of plant development, including strong root development and fruit/grain development, and even stress tolerance [53–55]. For example, K deficiency has been reported to induce PUT accumulation, but did not influence SPD and SPM contents in barley [56]. PUT accumulation has several roles in K deficiency, for example, plays a role in cation and osmolyte balance, pH regulation, ROS scavenging, and signalling [57]. P deprivation also resulted in high PUT accumulation and relatively low SPD and SPM levels in rice suspension cells, which was accompanied by cell growth inhibition [58]. Indeed, the accumulation of PUT leading to a high PUT/(SPD + SPM) ratio may even result in plant growth inhibition and stress [59]. However, under the present conditions, the detected changes are the outcomes of sufficient N supply, and the availability of P and K did not further influence them, as supported by the cluster analysis. It is also possible that the form or the combination of N supply (NO_3^-, NH_4^+ or urea) has a greater effect on plant metabolism than the dose of K and/or P, and plants have species-dependent preferences, too. For example, maize, soybeans, and vegetables are nitrate-preferred crops [60].

Variance analysis also revealed that phenological stage was the dominant factor of variability across all traits. The fertilisation level had broad but selective impacts, and genotype played only a minor role. The strong and repeated significance of phenological stage x fertilisation level interactions highlights that fertilisation effects were strongly stage-dependent. The genotype effects were generally weak, supporting the hypothesis that genotypic differences became evident at particular developmental stages but did not persist across all traits [61]. However, it should also be mentioned that an interesting developmental stage-dependent difference was observed between the two maize hybrids. Namely, the positive correlation between PUT and NO_3 contents persisted longer, until the R1 stage in the FAO 490, which could be necessary to ensure N transport for an extended period, to allow a better maintenance of grain filling and the subsequent N accumulation in the seeds. This finding highlight that the maintenance of the metabolic stability in this genotype even later in development helps in N remobilisation and grain filling.

In conclusion, in the present study, it has been demonstrated that at the early developmental stages of maize, the PA synthesis and accumulation are dominant, and at the R stages, their catabolism comes to the fore. It has been confirmed that PA metabolism is indeed N supply-responsive, as N fertilisation could successfully activate the synthesis but inhibit the catabolism of PAs. The observed changes were developmental stage- and N supply-dependent throughout the experiment. Based on the obtained results, PA metabolism is primarily determined by N supply, while the balanced N treatments have no significant further modulating effects on PA metabolism. The results of this study also confirmed that PUT and SPD may act as N metabolic buffer during grain filling, and help the plants to efficiently mobilise N flow from the leaves to the grains. The persistence of the correlation between PUT and NO_3 contents in the FAO 490 maize hybrid may suggest that the higher metabolic activity of the leaves can contribute to extra yield in the NPK experiment. However, this is not manifested in higher grain N content, which indicates the classic dilution effect, confirming the better N use efficiency of the genotype. Based on the authors' findings, it is important to highlight that the developmental stage determines the extent of nutrient responsiveness, which underscores the importance of synchronising nutrient management with crop developmental stages. Although these results were obtained under a one-year field experiment on two maize hybrids, the data provide a good basis for future experiments, where multiple genotypes can be used. It is reflected that it is sufficient to monitor the investigated parameters only up to the R1 stage to predict higher and/or balanced grain filling and N assimilation rate. For further investigations, the expression level of PA metabolism-related genes would also be recommended to reveal the genetic background of the hybrids.

CRediT authorship contribution statement

Magda Pál: Writing – original draft, Visualization, Validation, Supervision, Software, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Árpád Illés:** Writing – original draft, Visualization, Validation, Software, Methodology, Investigation, Formal analysis, Data curation. **Gabriella Szalai:** Writing – review & editing, Methodology, Formal analysis, Conceptualization. **Adrienn Széles:** Writing – review & editing, Supervision, Methodology, Investigation. **János Nagy:** Writing – review & editing, Supervision, Conceptualization. **Csaba Bojtor:** Writing – original draft, Visualization, Software, Methodology, Investigation, Formal analysis, Data curation.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jafr.2026.102949.

Data availability

Data will be made available on request.

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