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Effect of pre-harvest nitrogen on hormone profiles and dormancy
break in potato tubers

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Future Food Sustainability

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the degree of Future Food Sustainability MSc

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Abstract

This study tested whether pre-harvest nitrogen (N) supply alters the timing of dormancy release and the profiles of abscisic acid (ABA) and cytokinins (CKs) in apical buds of potato tubers. A field experiment was conducted with two cultivars, Brooke and VR808, receiving standard (100% N) and reduced (30% N) doses. After harvest, tubers were stored and assessed weekly for dormancy followed by collection of apical buds for hormone profiling and subsequent analysis using Liquid Chromatography–Tandem Mass Spectrometry (LC-MS/MS). Multiple PGRs were targeted; of these, only ABA and DZR were quantifiable and profiled across stages and treatments. Reduced nitrogen delayed dormancy break in both cultivars by around one week. Across the various dormancy stages, ABA in the apical buds remained stable and unaffected by nitrogen, indicating that a decline in ABA cannot always be associated with dormancy break. DZR showed cultivar- and nitrogen-dependent patterns, with shifts evident in Brooke but not in VR808, suggesting that cytokinin (CK) mobilisation could be genotype-conditioned. Overall, the findings suggest that N could modulate dormancy via altered ABA sensitivity rather than ABA decline, while DZR-linked cytokinin dynamics appear genotype-conditioned; this study also confirms the feasibility of nitrogen optimisation as an effective, environmentally friendly tool to extend dormancy, aid storage scheduling, and reduce fertiliser use.

Keywords: potato; dormancy break; nitrogen; abscisic acid; cytokinin; LC-MS/MS; storage.

Total word count - 6291

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Table of Contents

Academic integrity declaration.....	i
Abstract	ii
Acknowledgements	iii
List of Figures.....	v
1 Introduction.....	1
1.1 Dormancy in potato tubers.....	1
1.2 Nitrogen and its influence on potato dormancy	2
1.3 Phytohormonal control of dormancy	3
1.4 Nitrogen–hormone interactions: current understanding	4
1.5 Knowledge gaps and study rationale	4
1.6 Aim and objectives.....	5
2 Materials and methods	6
2.1 Plant material and experimental design	6
2.2 Tuber sampling and storage	6
2.3 Dormancy assessment	7
2.4 Plant growth regulators profiling	8
2.5 Sample preparation and extraction	8
2.6 Data analysis	9
3 Results	11
3.1 Dormancy break assessment	11
3.2 Hormone profiles.....	12
4 Discussion	14
4.1 Reduced nitrogen delayed dormancy break: insights from morphology-based staging.....	14
4.2 ABA in buds remained stable.....	16
4.3 DZR in buds showed cultivar and nitrogen-effects.....	18
4.4 Small bud-sample size constrained hormone detection but guides future improvements	20
References	23
Appendices.....	31
Appendix A - Ethical approval letter	31

List of Figures

Fig. 1. Schematic diagram of the split-plot field layout.	6
Fig. 2. Dormancy status categories in potato apical buds: (A) dormant (D), (B) pre-eye movement (PEM), (C) dormancy break (DB), (D) sprouted (small [SS], medium [MS], big [BS]). Adapted from Morris et al. (2018).....	7
Fig. 3. Dormancy break (%) over days after harvest (DAH) under Standard and Reduced nitrogen. Left: VR808 and Right: Brooke. Points are means; lines aid visualisation. LSD ($P \leq 0.05$) shown where significant.	11
Fig. 4. Absciscic acid (ABA; $\log_{10}$ -transformed, ng g^{-1} DW) in apical buds across dormancy stages under standard and reduced nitrogen. Left: VR808; right: Brooke. Bars show mean $\pm$ SD ($n = 5$). Stages: D = Dormant; DB = Dormancy Break; S = Sprouting	12
Fig. 5. Dihydrozeatin riboside (DZR; $\log_{10}$ -transformed, ng g^{-1} DW) in apical buds across dormancy stages under standard and reduced nitrogen. Left: VR808; right: Brooke. Bars show mean $\pm$ SD ($n = 5$). Stages: D = Dormant; DB = Dormancy Break; S = Sprouting	13

1 Introduction

Potato is one of the most important global food crops, with production reaching around 383 million tonnes in 2023 (FAO, 2024). Asia and Europe lead global potato production, with the UK producing approximately 4.7 million tonnes of potatoes (FAO, 2024; FAO–OWID, 2025). In the UK, potatoes are usually harvested once per year, making long-term storage essential to ensure a continuous supply throughout the year. Approximately 50 % of the UK's potato harvest is stored for up to 11 months (Alamar et al., 2017). While this ensures year-round supply, it also introduces the potential for food loss and waste (FLW). A recent UK-wide study by Gage et al. (2024) estimated that approximately 650 kilo tonnes of potatoes are lost or wasted annually, with around 7.1 % of this occurring during storage. Premature dormancy break and sprouting were identified as one of the major biological causes of these losses (Gage et al., 2024).

Chlorpropham (CIPC) was one of the sprout suppressants used during extended storage periods in the UK. However, it was banned in the UK and EU in 2019, challenging existing storage management practices (European Commission, 2019). This ban has created an urgent need among researchers to explore alternative means of extending tuber dormancy. Earlier researchers have explored several strategies, including optimising storage conditions (Murigi et al., 2021), externally applying plant growth regulators (PGRs) (Moletsane et al., 2022), and breeding potato cultivars with naturally longer dormancy durations (Sharma et al., 2021). Recent studies have also started to explore how pre-harvest factors—such as nutrient availability, in particular nitrogen management—affect dormancy duration (Zhang et al., 2021).

1.1 Dormancy in potato tubers

Dormancy in plants is defined as a temporary pause in the growth of any organ containing a meristem and is associated with reduced biological activity, hormonal rebalancing, and metabolic changes (Herrera et al., 2022). In potatoes, tuber dormancy refers to a physiological state in which the tuber itself does not

initiate sprouting even under conditions conducive for sprouting (15–20 °C; ~90% RH) (Liu et al., 2015). As adapted from general plant physiology, tuber dormancy is mainly classified into three types, which may occur sequentially or overlap: endodormancy (inhibition by internal factors); paradormancy (inhibition by signals from other parts of the tuber); and ecodormancy (external conditions prevent dormancy break) (Dogramaci et al., 2024). The duration of dormancy in potatoes is mainly influenced by cultivar genetics, internal physiological status—especially phytohormone balance—and storage conditions (Sonnewald and Sonnewald, 2014; Suttle, 2004; Di et al., 2024). Internal physiological status is, in turn, affected by multiple environmental factors, including temperature, humidity, light exposure during storage, and pre-harvest agronomic practices such as nutrient management (Muthoni et al., 2014). Previously, dormancy was thought to begin at harvest (Suttle, 2004). However, it more accurately starts during tuber development, particularly at the tuber initiation stage (Claassens & Vreugdenhil, 2000; Van Ittersum et al., 1992b), and is gradually established in the buds, starting from the stolon end and progressing towards the apical eye (Claassens & Vreugdenhil, 2000). Accordingly, regulation resides in the eye meristems—especially the apical bud at release (Sonnewald & Sonnewald, 2014).

Traditionally, dormancy break in potato tubers is defined as the point when at least 80 % of the tubers in a sample exhibit sprouts ≥ 2 mm in length (Van Ittersum et al., 1992b; Muthoni et al., 2014; Zhang et al., 2021). However, Morris et al. (2018) proposed a five-stage developmental scale in which dormancy break is defined at stage 3 (eye movement), where very small white buds (< 1 mm) become visible under a stereomicroscope, while sprouts ≥ 2 mm correspond to stage 5 (sprout stage), indicating post-dormancy growth (See Fig. 2).

1.2 Nitrogen and its influence on potato dormancy

Nitrogen is a key nutrient in potato cultivation, essential throughout development, especially during the vegetative and tuber bulking stages (Zhang et al., 2021; Di et al., 2024). Applied in the vegetative stage, nitrogen delays tuber initiation and bulking by promoting excessive shoot growth (Van Ittersum et al., 1992; Zhang et al., 2021). During the tuber bulking stage, nitrogen delays tuber maturity,

promotes the growth of secondary tubers and reduces tuber density (as reviewed in Alva, 2004). Therefore, tuber dormancy is likely influenced by pre-harvest conditions, including nitrogen-driven effects on development and maturity (Claassens & Vreugdenhil, 2000; Zhang et al., 2021).

1.3 Phytohormonal control of dormancy

Dormancy in tubers is closely linked to phytohormones. The onset, maintenance, and release of dormancy are influenced by the balance of several plant growth regulators (PGRs), including abscisic acid (ABA), auxins, cytokinins (CKs), gibberellins (GAs), ethylene and strigolactones (SLs) (Di et al., 2024; Dogramaci et al., 2024), together with carbohydrates and organic acids (Suttle, 2004). Ethylene is required for tuber initiation (Suttle, 1998a). ABA, on the other hand, is crucial for inducing and maintaining dormancy (Mani et al., 2014). In bulk parenchyma, ABA levels are found to decrease as endodormancy weakens; however, no definitive threshold concentration for dormancy release has been identified (Alamar et al., 2017; Destefano-Beltrán et al., 2006; Ordaz-Ortiz et al., 2015). Bud-focused studies are fewer and report more variable trajectories (Destefano-Beltrán et al., 2006; Sorce et al., 2005; Morris et al., 2018). ABA also interacts with other phytohormones and sugar metabolism pathways to facilitate dormancy release and sprouting (Destefano-Beltrán et al., 2006; Haider et al., 2022; Tsai & Gazzarrini, 2014). Auxins help re-establish connectivity between storage parenchyma and the apical bud, facilitating sucrose transport and sprouting (Debast et al., 2011; Tsai & Gazzarrini, 2014). CKs and GAs are involved in the reactivation of meristematic activity and sprout growth (Hartmann et al., 2011;). CK levels are found to rise in apical buds just prior to dormancy release, acting as key drivers of meristem reactivation (Morris et al., 2018). CKs also work synergistically with auxins and gibberellic acid (GA) to support sprout elongation (Aksenova et al., 2013). Meanwhile, GAs have been found to gradually increase during storage and are associated with enhanced sprout vigour (Roumeliotis et al., 2012). From past studies it is clear that hormone levels in the apical bud vary across storage time. However, fewer studies have explored how

pre-harvest nitrogen application influences these changes and the physiological control of tuber dormancy.

1.4 Nitrogen–hormone interactions: current understanding

Nitrogen, dormancy and phytohormones are interlinked, and several researchers have tried to explore this. Van Ittersum (1992b) found that excess nitrogen applied during the tuber bulking stage shortened dormancy by 5–8 days (dormancy break = visible sprouts ≥ 2 mm). Other studies have examined nitrogen-induced hormonal changes and their effects on dormancy. Zhang et al. (2021) tested three commercial potato cultivars (E shu3, Z shu5, and H shu3) under 0, 210, and 315 kg N ha⁻¹, storing tubers at 25 °C and sampling over 16 weeks. They found that higher nitrogen reduced dormancy by 3–6 weeks, promoted earlier sprouting, with associated GA increases at week 4, suggesting changes in hormonal and metabolic regulation. However, dormancy break was defined as appearance of visible sprouts (≥ 2 mm), which, according to Morris et al. (2018), corresponds to a sprouting stage rather than dormancy break.

1.5 Knowledge gaps and study rationale

Nitrogen's influence on dormancy duration is already known, with studies showing that reduced nitrogen extends it. But how each of these studies defines dormancy varies, with some defining its start at tuber initiation and others at harvest, and many defining dormancy break as the first ≥ 2 mm sprout. Most hormone profile studies come from bulk tissues (cortex, periderm) rather than apical buds, the major point of dormancy control. Existing nitrogen–hormone studies tend to sample PGRs that are less relevant for dormancy break (e.g. GA by Zhang et al., 2021) and that too at only a single point (e.g. dormant stage), missing what happens just before, at and after true release. As a result, there is a lack of bud-level studies on how different nitrogen rates influence the dynamics of PGRs more relevant to dormancy break such as ABA, CK, and IAA throughout dormancy progression and how these dynamics affect dormancy release.

1.6 Aim and objectives

This study aims to investigate how pre-harvest nitrogen fertilisation influences dormancy break and associated bud-level hormone dynamics in potato tubers. Specifically, it seeks to quantify hormone profiles (ABA, CK, and IAA levels) of two commercial cultivars (Brooke and VR808) in apical buds across storage and assess how nitrogen rates and dormancy stages affect PGR dynamics and dormancy progression. Additionally, it evaluates whether alternative dormancy definitions (different start and end points) change the interpretation of nitrogen's effect on time to dormancy break. It is hypothesised that reduced nitrogen will slow the rate of decline of ABA, delay the rise in CK and IAA in apical buds. The timing and magnitude of these changes are expected to vary across different nitrogen rates and cultivars, affecting the time to dormancy break in potato tubers.

2 Materials and methods

2.1 Plant material and experimental design

The field experiment was conducted during 2022–23 on a commercial farm in Nottinghamshire (coordinates- 53°06'43"N, 001°01'24"W). The experiment followed a split-plot design with cultivar ('Brooke' and 'VR808') as the main plot factor and replicate as the subplot factor. Each nitrogen treatment was randomly assigned to plots within each subplot (See Fig. 1.). Each variety had a different standard nitrogen application rate based on commercial practice. Brooke received a total of 190 kg ha⁻¹, while the standard application for VR808 was 230 kg ha⁻¹. For the reduced applications, Brooke received 130 kg ha⁻¹, while the reduced N application for VR808 was 157 kg ha⁻¹. 75% of the total N for each of these applications was applied at planting and the remaining 25% was applied at plant emergence. Tubers were grown at a seed spacing of 30 cm, a depth of 20 cm, and with a row spacing of 50 cm.

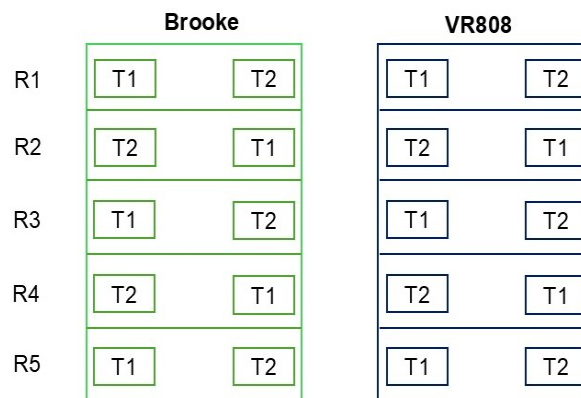


Fig. 1. Schematic diagram of the split-plot field layout.

2.2 Tuber sampling and storage

Tubers were harvested at commercial maturity using a digging fork. To avoid the edge effect, samples were taken from the central area of each subplot. Healthy tubers of uniform size between 40 and 70 mm with no visible damage were selected, bagged and transported to Cranfield University (25 °C, 1.5 hrs) for curing. During curing, tubers were kept at 15 °C, relative humidity (RH) > 80%, and in the dark for three days. After curing, tubers were stored under the same

temperature and humidity conditions. These tubers were later used for dormancy assessment and quantification of plant growth regulators (PGRs).

2.3 Dormancy assessment

The dormancy assessment was carried out according to Morris et al. (2018). In short, tuber dormancy and sprouting were assessed weekly under a stereomicroscope by observing the morphological development of the main bud from the apical eye using six stages (See Fig. 2). These stages include: dormant (D), where the meristem is fully covered by primordial leaves; pre-eye movement (PEM), where the meristem begins to emerge; dormancy break (DB), defined by the initial emergence of a sprout; small sprout (SS), when small white buds (< 1 mm) become visible; medium sprout (MS), with sprouts of 1–2 mm; and big sprout (BS), with sprouts > 2 mm. Assessments were carried out at eight sampling points following the curing process until dormancy was broken in all the treatments. At each time point, for each cultivar × nitrogen treatment, 10 tubers were assessed in each of five replicates (n = 5). Dormancy break for a replicate was recorded when ≥50% of its 10 tubers had reached DB or later (DB, SS, MS, or BS). Additionally, at each time point, from each replicate, five more tubers were collected; three apical buds per tuber (15 per replicate) were pooled within replicate, snap-frozen, freeze-dried, and stored at –80 °C until further PGR analysis.

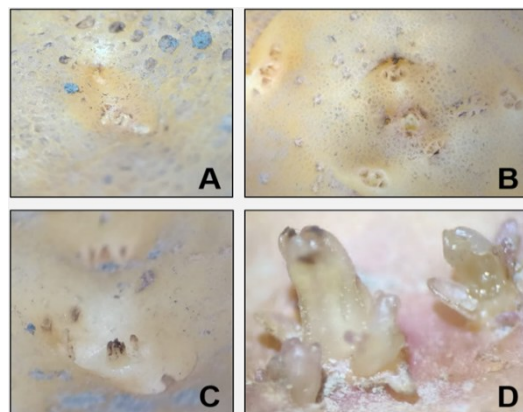


Fig. 2. Dormancy status categories in potato apical buds: (A) dormant (D), (B) pre-eye movement (PEM), (C) dormancy break (DB), (D) sprouted (small [SS], medium [MS], big [BS]). Adapted from Morris et al. (2018).

2.4 Plant growth regulators profiling

The PGRs targeted were abscisic acid (ABA) and its metabolites (dihydrophaseic acid (DPA), phaseic acid (PA), auxins (indole-3-acetic acid), and cytokinins (trans-zeatin(tZ), cis-zeatin(cZ), isopentenyladenine (iP), DHZ (dihydrozeatin) and their conjugated forms (iPR, tZR, cZR, DZR). All the standards and deuterated internal standards (iSTDs) were sourced from the National Research Council of Canada – Plant Biotechnology Institute, Olchemim (Czech Republic), and Sigma-Aldrich (UK).

2.5 Sample preparation and extraction

Plant hormones were extracted and quantified according to Tosetti et al. (2020) with some modifications. Approximately 0.1–3 mg of freeze-dried apical bud samples was weighed into 2 mL screw-cap tubes with two glass balls, followed by grinding using a star beater (VWR, UK) at 20 Hz for 1 minute. Following this procedure, 50 μ L of internal standard (100 ng mL⁻¹) was added, each containing the following deuterium-labelled compounds: [²H₃]dihydrozeatin (d3-DZ), [²H₃]dihydrozeatin riboside (d3-DZR), [²H₃]dihydrozeatin-9-glucoside (d3-DZ9G), [²H₅]trans-zeatin (d5-tZ), [²H₅]trans-zeatin riboside (d5-tZR), [²H₅]trans-zeatin riboside-5'-monophosphate sodium salt (d5-tZRP), [²H₅]trans-zeatin-7-glucoside (d5-tZ7G), [²H₅]trans-zeatin-9-glucoside (d5-tZ9G), [²H₅]trans-zeatin-O-glucoside (d5-tZOG), [²H₅]trans-zeatin-O-glucoside riboside (d5-tZROG), [²H₆]N⁶-isopentenyladenine (d6-IP), [²H₆]N⁶-isopentenyladenosine (d6-IPR), [²H₆]N⁶-isopentenyladenosine-5'-monophosphate sodium salt (d6-IPRP), [²H₆]N⁶-isopentenyladenine-7-glucoside (d6-IP7G), [²H₆]N⁶-isopentenyladenine-9-glucoside (d6-IP9G), [²H₃]dihydrophaseic acid (d3-DPA), [²H₅]abscisic acid glucose ester (d5-ABA-GE), [²H₃]phaseic acid (d3-PA), [²H₄]7'-hydroxy-abscisic acid (d4-OH-ABA), [²H₄]abscisic acid (d4-ABA), [²H₅]indole-3-acetic acid (d5-IAA), and [²H₅]indole-3-acetylaspatic acid (d5-IAA_{sp}). Subsequently, 950 μ L of cold extraction solvent (methanol:formic acid:water, 60:5:35, v/v/v), containing 250 mg L⁻¹ BHT, was added. This mixture was homogenised in the star beater again at 20 Hz for 1 minute. The samples were then incubated on ice and in the dark for 20 minutes, followed by centrifugation at 12,000 RCF at 4 °C for 10

minutes. The resulting supernatant was pipetted into a new set of amber centrifuge tubes and then snap-frozen and freeze-dried using a solvent freeze drier overnight. Samples were then reconstituted in 500 μL of methanol:water :1 mM ammonium formate (50:50, v/v) mix, vortexed for 10 seconds, then sonicated on ice for 2 minutes. Samples were then filtered using 0.20 μm , 4 mm diameter syringe nylon filters into amber LC-MS vials with deactivated glass inserts. Extracts were stored at $-80\text{ }^{\circ}\text{C}$ prior to LC-MS/MS analysis. Each extraction batch consisted of 22 biological samples, a blank (containing internal standards and extraction solvent), and a double blank (containing extraction solvent only) to assess potential contamination, background signals, and recovery.

Quantification of phytohormones was performed according to Tosetti et al. (2020) with modifications to the LC conditions. Analyses were performed on an LC-MS/MS system, consisting of an Agilent 1200 series HPLC (Agilent, Berks., UK) coupled to a Q-Trap 6500 mass spectrometer (AB Sciex, MA, USA). The prepared extracts were injected onto a Hypersil GOLD C18 column ($50 \times 2.1\text{ mm}$, $1.9\text{ }\mu\text{m}$) with a guard at $30\text{ }^{\circ}\text{C}$. Mobile phase A was water with 0.1% formic acid and 1 mM ammonium formate; mobile phase B was methanol with 0.1% formic acid and 1 mM ammonium formate. Separation used a fast gradient at 0.40 mL min^{-1} : 0–1.0 min, 95% A / 5% B; 5.0 min, 60/40; 6.5–7.5 min, 5/95; 7.6–8.5 min, 95/5. Injection volume $10\text{ }\mu\text{L}$. Calibration curves were prepared using ten concentrations (0.1, 0.25, 0.50, 1.0, 10, 25, 50, 100, 150, 250- ng mL^{-1}) of known standards of abscisic acid (ABA), cytokinins (CKs), and indole-3-acetic acid (IAA). PGR concentrations were determined by comparing the peak areas of the samples with those of known standards, using the ratio of the sample signal to the internal standard signal. Only calibration curves with $R^2 > 0.95$ were accepted.

2.6 Data analysis

Dormancy-break data were calculated as the percentage of tubers per replicate that had reached DB or later stages (DB, SS, MS, BS) at each sampling point. Replicate percentages were then averaged across replicates to obtain treatment means. Dormancy-break data met ANOVA assumptions without transformation;

thereafter, a repeated-measures ANOVA was performed. Pairwise differences were tested with Fisher's least significant difference (LSD, $\alpha = 0.05$).

Raw data from the LC-MS/MS were normalised to ng g^{-1} DW by considering the weight of the sample used for extraction. PGR concentration data were first analysed for normality using the Shapiro–Wilk test; however, the results remained non-normal. This was followed by removal of outliers and transformation using $\log_{10}$, which satisfied the normality and assumptions of ANOVA. A general ANOVA was then performed on the transformed data.

Even though there were eight sampling points throughout storage, only three sampling points—dormant (D), dormancy break (DB), and sprouting (S)—were taken into consideration (13, 20, and 62 days after harvest (DAH) for VR808 and 13, 34, and 55 DAH for Brooke). The choice of these points was due to their ability to best capture the physiological transition from deep dormancy to active sprouting. All statistical analyses were conducted on these representative stages. All statistical analyses were performed in GenStat (Version 21).

3 Results

3.1 Dormancy break assessment

The proportion of tubers that had reached dormancy break increased over time in both cultivars under both nitrogen treatments (See Fig. 3).

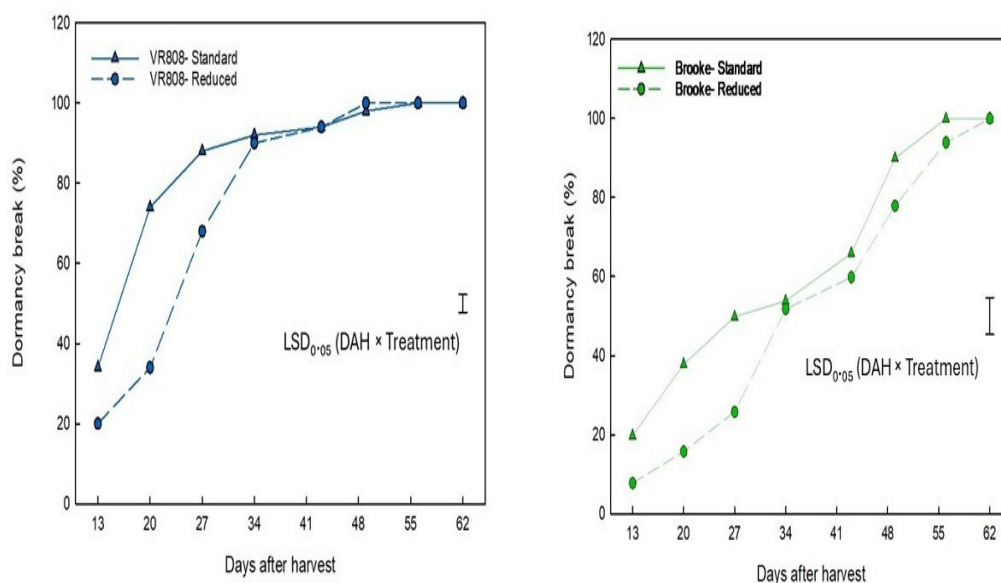


Fig. 3. Dormancy break (%) over days after harvest (DAH) under Standard and Reduced nitrogen. Left: VR808 and Right: Brooke. Points are means; lines aid visualisation. LSD ($P \leq 0.05$) shown where significant.

In VR808, dormancy break occurred under standard nitrogen between 13 and 20 DAH, while under reduced nitrogen it occurred between 20 and 27 DAH, showing approximately a one-week delay. Significant effect of nitrogen was seen up to 27 DAH; after that, no significant differences were observed ($p \geq 0.05$). Both treatments converged by 34 DAH, reaching ~100% by 49–56 DAH.

In Brooke, dormancy break was slower compared with VR808. Dormancy break occurred at 27 DAH under standard nitrogen and between 27 and 34 DAH under reduced nitrogen, showing almost a one-week delay. No significant nitrogen effects were observed after 27 DAH. Both treatments reached 100% dormancy break by 62 DAH.

Overall, VR808 reached dormancy break faster than Brooke, and nitrogen effects were observed mainly in the earlier stages of storage.

3.2 Hormone profiles

Among the targeted hormones (ABA, DPA, PA, IAA, DZR, tZ, tZR, cZ, iPR, iPRP), only abscisic acid (ABA) and dihydrozeatin riboside (DZR) were quantified at dormant (D), dormancy-break (DB), and sprouting (S) stages of the tubers. The remaining analytes were detected intermittently but were below the limit of quantification (<LOQ) in most replicates, so they were not analysed further. PGR data were analysed and presented on a $\log_{10}$ scale, with histograms displaying error bars ($\pm$ SD) (raw data are provided in Appendix B).

ABA in both Brooke and VR808 was stable across the three stages, i.e. dormant, dormancy break, and sprouting, and was unaffected by nitrogen ($p > 0.05$ for stage, nitrogen, and stage $\times$ nitrogen interaction) (see Fig. 4).

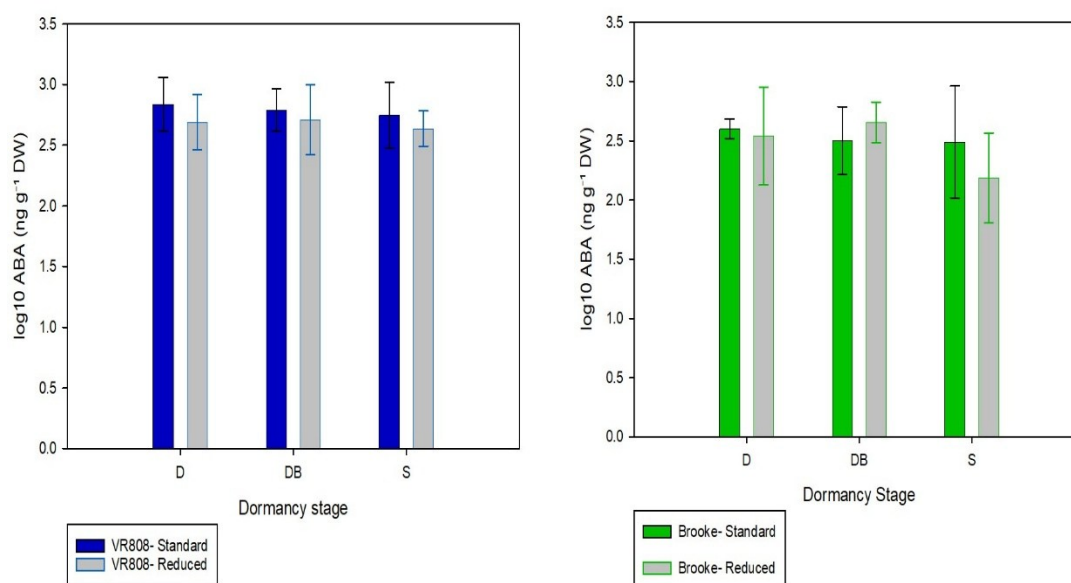


Fig. 4. Absciscic acid (ABA; $\log_{10}$ -transformed, ng g⁻¹ DW) in apical buds across dormancy stages under standard and reduced nitrogen. Left: VR808; right: Brooke. Bars show mean $\pm$ SD ($n = 5$). Stages: D = Dormant; DB = Dormancy Break; S = Sprouting

For DZR in Brooke, there were significant differences between dormancy stages, nitrogen, and their interactions ($P < 0.01$). Under standard nitrogen, DZR levels declined from dormancy-to-dormancy break before rising again at the sprouting stage. In contrast, under reduced nitrogen, they increased consistently up to sprouting, with values remaining above those under standard nitrogen at each point. In VR808 tubers, however, no significant differences in DZR content were found among dormancy stages, nitrogen treatments, or their interaction ($p > 0.05$) (see Fig. 5).

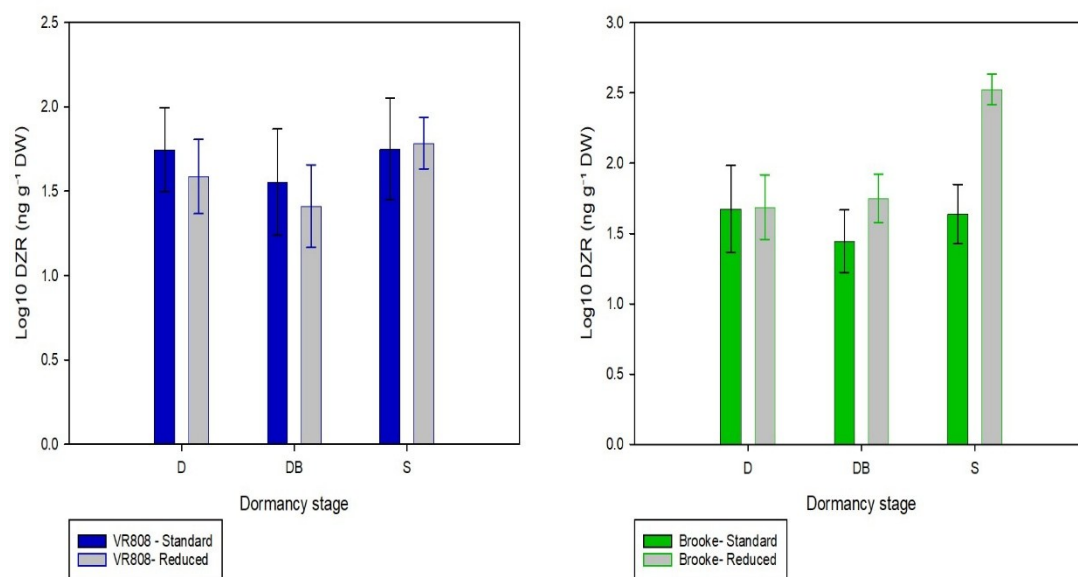


Fig. 5. Dihydrozeatin riboside (DZR; $\log_{10}$ -transformed, ng g^{-1} DW) in apical buds across dormancy stages under standard and reduced nitrogen. Left: VR808; right: Brooke. Bars show mean $\pm$ SD ($n = 5$). Stages: D = Dormant; DB = Dormancy Break; S = Sprouting

Overall, the levels of DZR were cultivar dependent, with nitrogen showing an effect on Brooke but not on VR808.

Graphs of untransformed data (ABA and DZR) are provided in the Appendix B.

4 Discussion

4.1 Reduced nitrogen delayed dormancy break: insights from morphology-based staging

The results of the present study show that reduced nitrogen delayed dormancy break by around one week in both Brooke and VR808 (measured in days after harvest using bud morphology-based staging).

This agrees with previous studies, such as Van Ittersum et al.(1992b), which reported that increased nitrogen application at planting shortened dormancy duration, and argued it was likely due to shifts in tuber-development timing (e.g., delayed tuber initiation). Zhang et al. (2021) found that reducing N extended dormancy by 3–6 weeks and was associated with reduced GA₁, sucrose, and α -amylase activity in buds, linking them to tuber development. However, the present study argues that nitrogen's effect on dormancy duration is more likely mediated by hormone profiles, specifically ABA and CK dynamics at dormancy break (possibly reflecting nitrogen-driven effects on development and maturity) (see Sections 4.2 and 4.3).

While previous studies show nitrogen's effect on dormancy duration, most of them have equated dormancy break with sprouting, i.e. the emergence of a sprout > 2 mm in length (Van Ittersum et al.,1992b; Zhang et al., 2021). This approach has several problems. Firstly, dormancy break occurs before the sprout reaches 2 mm, so it does not show the exact time point of physiological loss of dormancy (Claassens & Vreugdenhil, 2000). Secondly, nitrogen is a mineral nutrient that has been shown to influence sprout vigour and length (Zarzyńska et al., 2022). Thirdly, some cultivars show much greater sprout elongation behaviour, with certain genotypes sprouting far faster than others under identical storage conditions (Zou et al., 2024). In addition, sprout elongation is highly sensitive to environmental factors such as temperature, humidity, and light, which act independently of the dormancy state (Di et al., 2024).

All these factors create the possibility that part of any treatment's effect (especially nitrogen's) on dormancy in earlier studies reflects differences in post-

dormancy growth rate rather than the actual dormancy break. Most importantly, this criterion ignores the hormonal and metabolic events that occur at the time of dormancy release and between dormancy release and sprouting. Only a limited number of studies have properly distinguished between these stages (Morris et al., 2018; Tosetti et al., 2020), while others discuss them almost interchangeably (Zhang et al., 2021; Van Dingenen et al., 2019). The use of bud-morphology-based scoring with hormone profiling in the present study helps capture this critical physiological transition more precisely. Therefore, this is a critical gap in the literature, and, in the future, more studies need to be conducted in this area to understand how sugars, genetics, and PGRs interact with each other to provide a better physiological understanding of dormancy release.

Another unresolved issue in the literature is around dormancy duration and how it is defined. Van Ittersum et al.(1992b), for example, defined dormancy duration as the number of days after tuber initiation until a 2 mm sprout emerged, reasoning that dormancy induction occurs at tuber initiation. However, this definition remains debated, as other researchers argue that tuber dormancy begins at harvest (Suttle, 2004). The difficulty in adopting the former metric is that it is very hard to determine the exact time of tuber initiation, and it is strongly influenced by field conditions such as nitrogen, temperature, and photoperiod. For example, in the van ittersum study, tuber initiation was defined as the day when at least six plants had been sampled and more than half of their stolons showed swelling at least twice the stolon diameter (Van Ittersum et al.,1992b); this metric is difficult to standardise across experiments. In contrast, a days-after-harvest metric is easier to standardise and measure, has direct relevance for storage and industry, and reliably captures the physiological processes of dormancy release and sprouting, including the associated hormonal and metabolic dynamics (Morris et al., 2018), during the storage period itself. Thus, measuring from harvest is the more practical and physiologically meaningful definition for both research and application.

Overall, reduced N delayed physiological dormancy break by about one week in both cultivars. The use of stage-resolved morphology separates DB from

sprouting and clarifies treatment effects more precisely, whereas using days-from-initiation rather than days-after-harvest does not change the interpretation of treatments.

4.2 ABA in buds remained stable

In this study, ABA levels were stable across storage, with only minor, statistically non-significant changes across dormancy, dormancy break, and sprouting, and no statistically significant differences were detected between standard and reduced nitrogen treatments.

This contrasts with previous studies, which reported a consistent decline in ABA throughout the storage period and associated it with dormancy break (Sonnewald, 2014; Foukaraki et al., 2015). Therefore, this study does not support the hypothesis that ABA decline is associated with dormancy break. However, it could still support the idea that no threshold ABA concentration is necessary for dormancy break to occur (Destefano-Beltrán et al., 2006; Ordaz-Ortiz et al., 2015).

Most studies that observed a consistent decline of ABA during storage were conducted on tissues such as the periderm and cortex (Suttle, 2004; Mani et al., 2014). On the other hand, studies conducted on apical buds showed a more complex pattern (e.g., Destefano-Beltrán et al., 2006; Morris et al., 2018; Sorce et al., 2005). Destefano-Beltrán et al. (2006) and Morris et al. (2018) reported that ABA increased up to mid-storage and then declined, whereas Sorce et al. (2005) found that ABA concentration increased in the buds until sprouting, followed by a decline, while bulk-tissue assays in the same study showed the opposite trend. Taken together, these results suggest that ABA decline in buds during storage is not always associated with the end of tuber dormancy and that the ABA profile could be cultivar- and tissue-specific.

Studies examining the effect of nitrogen on ABA are limited and only provide partial insight. Van Dingenen et al. (2019) tested different nitrogen application rates on potatoes and measured ABA at harvest, reporting lower ABA under reduced nitrogen compared with standard supply, although there were cultivar

differences in ABA concentration. Zhang et al. (2021) measured hormones in apical buds at the dormant stage and reported that higher nitrogen increased ABA, GA₁, sucrose, and α -amylase activity, coinciding with earlier sprouting. However, both of these studies measured hormones only at the dormant stage and do not provide information on ABA levels at dormancy break and sprout stages. Since there are no previous studies that profile ABA at dormancy break and sprout stages under reduced nitrogen, the present results suggest that dormancy release under reduced nitrogen could occur independently of ABA concentration.

One possible biological explanation for the dissociation between dormancy break and ABA decline in the present study could be that dormancy release depends on ABA sensitivity rather than absolute ABA levels. High sucrose is known to interact with ABA signalling, with trehalose-6-phosphate (T6P) acting as a sucrose-derived signal that reduces ABA sensitivity (Tsai & Gazzarrini, 2014). Debast et al. (2011) showed that dormancy release and sprouting in potato were strongly associated with sucrose accumulation and carbohydrate status, which interact with ABA and GA metabolism. Tsai & Gazzarrini (2014), working in *Arabidopsis*, demonstrated that the T6P pathway functions as a key sucrose signal that antagonises ABA action by reducing ABA sensitivity. Similar effects have been seen in potato seedlings, where *StHAB1* expression modified ABA-dependent traits such as germination inhibition without changing ABA levels (Liu et al., 2023). Collectively, these studies support the possibility that dormancy transition could occur without significant changes in ABA concentrations.

This mechanism could also explain the delayed dormancy break under reduced nitrogen. Zhang et al. (2021) reported that high nitrogen increased sucrose and α -amylase activity, coinciding with earlier sprouting and higher ABA. Van Dingenen et al. (2019) demonstrated that limited nitrogen altered carbohydrate partitioning, leading to reduced sucrose content and delayed sprouting in several potato cultivars. Therefore, reduced N could lower sucrose/T6P, maintaining higher ABA sensitivity and delaying dormancy, whereas standard N would sustain sucrose/T6P and reduce ABA sensitivity. Although sucrose was not

measured in the present study, it is plausible that nitrogen affected sugar signalling in a way that influenced dormancy release independently of ABA concentration. Most importantly, dormancy is governed by the balance of various PGRs, including GA and CK, which promote sprouting, and auxins and strigolactones, which interact with ABA signalling (Roumeliotis et al., 2012; Alamar et al., 2017; Di et al., 2024). Overall, these possibilities highlight that the effect of nitrogen on dormancy may act through complex interactions between carbohydrate signals, multiple hormones, and gene expression, and may not depend on ABA concentration at all.

Taken together, the results show that reduced nitrogen delayed dormancy break without associated changes in ABA levels, contradicting this study's hypothesis that nitrogen acts by slowing ABA decline and indicating that ABA decline is not always linked to dormancy release.

4.3 DZR in buds showed cultivar and nitrogen-effects

In the present study, dihydrozeatin riboside (DZR) showed cultivar-specific patterns: in Brooke, it increased across storage and was higher under reduced N, whereas in VR808 it remained stable with no significant treatment effect, indicating that cytokinin dynamics are cultivar-dependent and N-responsive.

Previous studies have consistently associated cytokinins with dormancy break and subsequent sprouting (Suttle, 1998, 2008; Hartmann et al., 2011; Morris et al., 2018). Studies show that CK levels in apical buds increase at the onset of sprouting (Suttle, 1998b, 2008; Hartmann et al., 2011). In a study by Hartmann et al. (2011), transgenic suppression of CK by expression of a cytokinin oxidase/dehydrogenase gene delayed dormancy release, confirming that a minimum CK signal is required for this transition (dormancy break). More recent studies suggest that CKs may be more critical for regulating sprout vigour than for controlling the exact timing of dormancy break. For example, Morris et al. (2018) found that apical buds of *StCEN*-silenced lines accumulated higher levels of isopentenyl- and trans-zeatin-type CKs, which coincided with vigorous sprout growth, whereas overexpressing lines maintained lower CK levels and weaker sprouts. However, the timing of dormancy release was not altered in that study,

suggesting that CKs participate in the reactivation of meristematic activity, but their strongest influence may be on post-dormancy bud growth.

Most studies on potato PGRs focus on cytokinins such as tZ, cZ, iPRP, and iPR (Hartmann et al., 2011; Morris et al., 2018), while DZR has received little attention. Its biological role is still debated because riboside cytokinins are generally thought to be less active than the free bases, serving mainly as transport or storage forms (Vylíčilová et al., 2020). In potato, this idea is supported by the enzyme StCKP1, which converts ribosides like DZR into the free base DHZ, effectively making DZR a reserve that can be drawn upon when needed (Bromley et al., 2013). Consistent with this, potato CK receptors (StHK2/3/4) show high affinity for free bases—tZ and iP, and in particular DHZ through StHK3—rather than for ribosides (Lomin et al., 2018), suggesting that DZR itself is unlikely to act directly as a signalling molecule. Even so, evidence from other species indicates that DZR may still have developmental importance. In rose cuttings, for example, Otiende et al. (2021) observed that DZR levels rose after planting and peaked just before visible bud break, pointing to a possible role in bud activation. Taken together, these findings underline the uncertainty: DZR can show striking patterns around sprouting, but its exact function in potato dormancy release remains unresolved.

Building on the above, the DZR results are interpreted here by considering it as a storage form rather than an active CK. In Brooke, under standard N, the DZR dip at dormancy break could be associated with a conversion pulse (DZR → free-base CK) that triggers meristem reactivation, followed by restocking of the DZR reserve (Bromley et al., 2013). Under reduced N, the continuous DZR rise could suggest greater stocking without efficient conversion and/or weaker receptor drive (less activation of CK receptors by free-base CKs), aligning with delayed bud break (Bromley et al., 2013; Lomin et al., 2018). In VR808, a flat DZR profile could imply that DZR is not the controlling pool for this genotype; dormancy timing may depend more on other CK forms (tZ/iP free bases) (Bromley et al., 2013; Lomin et al., 2018). Future work should co-quantify DZR with free bases, assay riboside → free-base conversion, and profile receptor expression/affinity to test

whether DZR mobilisation translates into effective CK signalling at dormancy release (Bromley et al., 2013; Morris et al., 2018).

Hence, contrary to this study's hypothesis that reduced nitrogen would delay CK rise, DZR patterns instead revealed cultivar-specific and nitrogen-responsive dynamics, with their precise role in dormancy break remaining unresolved.

4.4 Small bud-sample size constrained hormone detection but guides future improvements

Among the targeted hormones (ABA, DPA, PA, IAA, DZR, tZ, tZR, cZ, iPR, iPRP), only ABA and DZR were consistently quantified at the dormant (D), dormancy-break (DB), and sprouting (S) stages; the remaining analytes were detected intermittently but were <LOQ in most replicates and were not analysed further. Using LC–MS/MS workflows similar to those applied in this study, Morris et al. (2018) quantified ABA and several cytokinins in apical buds (tZ, tZR, tZRP, iPR, iPRP), and Tosetti et al. (2020) quantified ABA and phaseic acid (PA) in cortex tissue (DPA not detected).

Since only few hormones were quantifiable in this study, it is also essential to consider potential methodological limitations. The extraction and quantification protocol were adapted from Morris et al. (2018), which had been validated for apical bud tissues in the 0.3–3 mg dry weight range. However, in the present work, many of the samples fell below 0.3 mg DW, i.e. outside the validated range. Moreover, apical tissues were sometimes transferred between tubes prior to extraction, which may have introduced additional handling losses.

At such low biomass, even minor errors can have disproportionately large effects on measured hormone concentrations (Šimura et al., 2018). Small handling errors during extraction could cause substantial variability in measured hormone concentrations, as has been noted more generally in studies working with very small tissue masses (Tarkowská et al., 2014b; Cao et al., 2020; Šimura et al., 2018). Thus, working below the validated tissue range in this study could have contributed to noise and inconsistency in the hormone data.

Another limitation arises from the matrix effect. Without thorough purification, extracts from potato buds typically contain pigments, phenolics, sugars, and other secondary metabolites in much higher abundance than hormones (Cao et al., 2020). These co-extracted compounds enter the electrospray source together with the analytes and compete for ionisation capacity, a process known as ion suppression. Under such conditions, the apparent signal of low-abundance hormones could be artificially reduced, even if the compounds are present in the tissue (Tarkowská et al., 2014; Cao et al., 2020). The inclusion of a solid-phase extraction (SPE) step has been shown to substantially reduce these matrix interferences by selectively retaining or washing away unwanted metabolites (Šimura et al., 2018). By contrast, the protocol employed here (Morris et al., 2018) used only physical filtration through 0.20 µm nylon membranes, which removes particulates but does not eliminate dissolved interfering compounds. This lighter purification may therefore have left significant background compounds in the extracts, increasing ion suppression and disproportionately obscuring hormones such as IAA that occur at extremely low endogenous levels.

The inability to quantify IAA across samples could be explained by solvent chemistry. The extraction solvent (MeOH/formic acid/H₂O, 60:5:35) is strongly acidic, a condition under which several IAA conjugates are unstable. Acidic extractions (pH < 3) have been shown to cause significant degradation of IAA conjugates, whereas neutral extraction with 50% aqueous acetonitrile preserves analyte stability (Šimura et al., 2018). Thus, even though the method included deuterated IAA as an internal standard, the endogenous free IAA pool may have degraded during sample preparation. It is also possible that the auxin present in the apical buds occurred mainly as other IAA conjugates (e.g. IAA–glucose, IAA–glutamate) outside the targeted panel, which would not have been quantified despite being physiologically relevant (Novák et al., 2012).

Thus, very small bud masses and limited clean-up constrain hormone sensitivity, so null effects should be interpreted cautiously; in particular, IAA could not be reliably quantified, meaning the hypothesis regarding reduced N delaying IAA rise could not be tested.

5. Conclusions

This study investigated whether pre-harvest nitrogen(N) application affects dormancy break and the associated hormone profiles in apical buds post-harvest. Across both cultivars, nitrogen reduction extended dormancy duration by almost one week. This shift occurred without a marked change in apical-bud ABA, which runs against the ABA-decline hypothesis and could be consistent with sensitivity-based rather than concentration-based ABA control. DZR exhibited cultivar-specific, nitrogen-responsive changes, suggesting that CK form and genetic background shape cytokinin dynamics in apical buds. The use of bud-level morphology allowed separation of dormancy break from sprouting and confirmed nitrogen's effect on the true physiological release of dormancy, indicating that the definition of 'dormancy break' matters for interpretation, as confusing it with sprouting may conceal true treatment effects.

This study has practical implications for stakeholders across the potato supply chain and for research and development (R&D). For growers and agronomists, a moderate reduction in pre-harvest nitrogen is an environmentally friendly way to extend tuber shelf life. For industry, reduced N helps maintain marketable quality for longer, supporting more consistent pack-out through the storage. For researchers, this reinforces that dormancy is more complex and is shaped by interacting hormones, sugars, gene expression, and bud biology. A practical next step would be to test the hypotheses put forward by this study across different cultivars and nitrogen regimes. Overall, optimising nitrogen rate is a practical, low-cost, environmentally friendly method for reducing storage losses and extending dormancy, but greater explanatory clarity will come from resolving genotype-dependent bud-level mechanisms of dormancy release

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Appendices

Appendix A - Ethical approval letter



31 May 2025

Dear Mr Muralidharan ,

Reference: CURES/25430/2025

Project ID: 28870

Title: Influence of Pre-Harvest Nitrogen Management on Plant Growth Regulator (PGR) Profiles of Potato Tubers

Thank you for your application to the Cranfield University Research Ethics System (CURES).

We are pleased to inform you your CURES application, reference CURES/25430/2025 has been reviewed. You may now proceed with the research activities you have sought approval for.

If you have any queries, please contact CURES Support.

We wish you every success with your project.

Regards,

CURES Team

Appendix - B

B.1 Hormone profiles (original scale)

Untransformed ABA concentrations ($\text{ng g}^{-1} \text{ DW}$) are shown for reference; normality was not met on the original scale, so $\log_{10}$ -transformed analyses are reported in the Results.

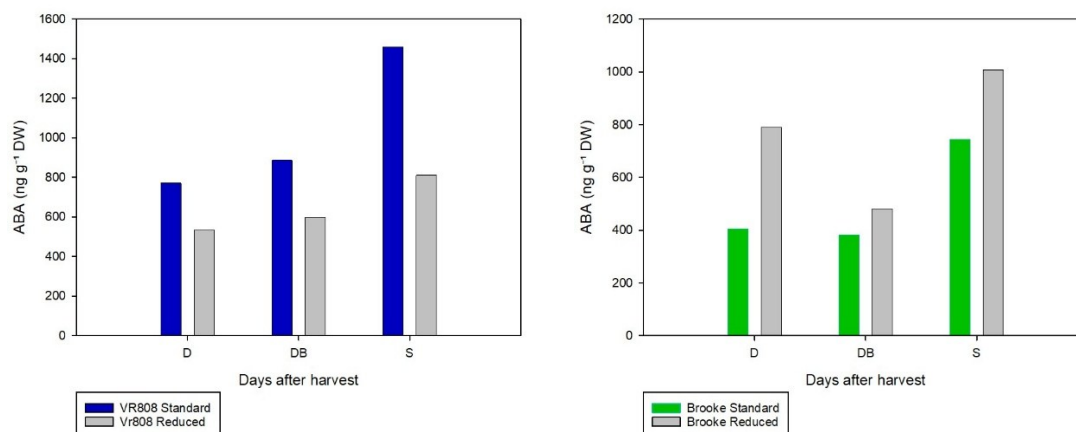


Fig. B.1 Original-scale ABA ($\text{ng g}^{-1} \text{ DW}$) in apical buds of Brooke and VR808 under 100% and 30% N at D, DB, and S; statistical analyses are reported in the Results ($\log_{10}$ -transformed).

For DZR, original-scale concentrations ($\text{ng g}^{-1} \text{ DW}$) are shown for reference; owing to non-normality on the raw scale, inference in the Results is based on $\log_{10}$ -transformed values.

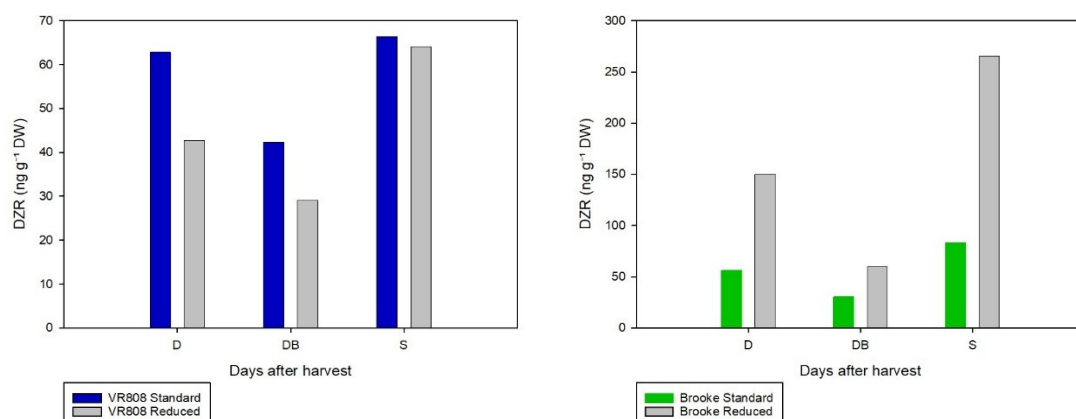


Fig. B. 2 Original-scale DZR ($\text{ng g}^{-1} \text{ DW}$) in apical buds of Brooke and VR808 under 100% and 30% N at D, DB, and S; statistical analyses are reported in the Results ($\log_{10}$ -transformed).

