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Physiological and biochemical changes during seed maturation and desiccation in soybean

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Abstract

Roughly 60% of soybean seed dry weight accumulates during the final three weeks before physiological maturity. The present research tracked physiological and biochemical changes from early seed fill (R5) through full maturity (R8) and post-maturity desiccation in two soybean cultivars, Awassa-95 and Belessa-95, grown at the Oromia Institute of Agricultural Sciences, Jimma, Ethiopia, during 2022. Moisture content declined from 57.3% at R5 to 12.8% at R8, while protein and lipid rose by 74% and 68%, respectively. ABA peaked at R7 and preceded sharp increases in LEA proteins, dehydrins, and antioxidant enzyme activities. SOD activity at R8 was 2.6-fold higher than at R5. Desiccation tolerance was acquired between R7 and R8, coinciding with maximum LEA protein accumulation. The findings confirm that the ABA-mediated protective pathway is central to desiccation tolerance acquisition in soybean seeds.

Keywords: Seed maturation, desiccation tolerance, soybean, physiological changes, seed development, abscisic acid, LEA proteins, antioxidant enzymes, seed moisture, germination viability

Introduction

An estimated 353 million tonnes of soybean were produced worldwide in 2022, making it the most important oilseed crop on earth ^[1]. Behind that statistic lies the transformation of a watery, metabolically active embryo into a dry, quiescent seed capable of surviving months of storage. Seed maturation in soybean spans several well-defined reproductive stages, from R5 to R8, during which moisture drops from above 55% to below 15% ^[2]. Protein and oil reserves accumulate rapidly, and a suite of protective molecules LEA proteins, dehydrins, and small heat shock proteins are synthesised under the control of abscisic acid ^[3].

Desiccation tolerance is acquired progressively during later maturation stages and reaches its maximum around R8 ^[4]. Seeds harvested prematurely show reduced germination and shorter storage life ^[5]. The biochemical basis involves LEA proteins preventing protein aggregation ^[6], non-reducing sugars replacing water molecules through vitrification ^[7], and antioxidant enzymes scavenging reactive oxygen species ^[8]. ABA orchestrates this protective response by activating transcription factors that switch on relevant gene sets ^[3].

Despite decades of research on model species, detailed time-course data on seed maturation in tropical soybean cultivars remain scarce, particularly for East African highland varieties ^[9], ^[10]. This research aimed to document the physiological and biochemical transitions from early seed fill through post-maturity desiccation and relate these changes to desiccation tolerance acquisition.

Research area description

The research was conducted at the experimental farm of the Oromia Institute of Agricultural Sciences near Jimma, Ethiopia (7°40'N, 36°50'E, elevation 1,753 m). The site has deep Nitisols with loamy clay texture, pH 5.3, and organic carbon 2.87%. The 2022 main season (June-November) received 1,482 mm of rainfall, with gradually declining rainfall during the R5-R8 period favouring seed drying on the plant.

Material and Methods

Material

Two soybean cultivars, Awassa-95 and Belessa-95, were used. Certified seed was obtained from the Jimma Agricultural Research Centre. Chemical reagents for protein and lipid

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determination were from Fisher Scientific. ABA was quantified by ELISA (Phytodetek, Agdia Inc.). LEA and dehydrin antibodies were from Agrisera AB. Antioxidant enzyme assay kits were from Cayman Chemical [11].

Methods

The experiment followed a randomised complete block design with five replications. Plots measured 4 m × 3 m; sowing was on 18 June 2022. Seeds were sampled at R5 (14 September), R6 (28 September), R7 (12 October), R8 (26 October), and post-R8 (9 November). Fifty seeds per plot were frozen in liquid nitrogen and stored at -80°C [12].

Results

Table 1: Physiological changes during seed maturation in soybean (mean of two cultivars)

Stage	Moisture (%)	Protein (% DW)	Lipid (% DW)	Sugars (mg g ⁻¹)	ABA (ng g ⁻¹)	Germ. after drying (%)
R5	57.3 ± 2.1a	22.1 ± 1.4d	11.3 ± 0.9d	38.7 ± 2.8a	15.2 ± 1.8d	8.3 ± 3.2d
R6	43.8 ± 1.7b	29.4 ± 1.2c	14.8 ± 0.7c	31.2 ± 2.3b	28.7 ± 2.4c	24.7 ± 5.1c
R7	28.4 ± 1.3c	34.6 ± 1.1b	17.5 ± 0.8b	26.8 ± 1.9c	42.3 ± 3.1b	61.3 ± 4.8b
R8	12.8 ± 0.9d	38.4 ± 0.9a	19.7 ± 0.6a	22.1 ± 1.7d	58.9 ± 3.7a	92.7 ± 2.1a
Post-R8	9.1 ± 0.7e	38.8 ± 1.0a	19.9 ± 0.7a	21.4 ± 1.5d	41.6 ± 2.9b	91.4 ± 2.8a

Values are means ± SE (n = 10). Different letters within columns differ at $p \leq 0.05$

Protein and lipid increased continuously from R5 to R8 then plateaued (Table 1). ABA peaked at R8 before declining at

Moisture was determined gravimetrically at 103°C for 72 hours [13]. Protein was estimated by micro-Kjeldahl ($N \times 6.25$), lipid by Soxhlet extraction. ABA was extracted with 80% methanol and quantified by ELISA. LEA proteins and dehydrins were analysed by immunoblotting; band intensities were quantified using ImageJ [14]. SOD, CAT, and APX were assayed spectrophotometrically [8]. Desiccation tolerance was assessed by drying seeds to 8% moisture over silica gel and evaluating germination at 25°C [15]. Data were analysed by ANOVA in R 4.2.1 with Fisher's LSD at $p \leq 0.05$.

post-R8. Germination after controlled desiccation climbed from 8.3% at R5 to 92.7% at R8.

Table 2: Antioxidant enzyme activities and protective protein levels during seed maturation

Stage	SOD (U mg ⁻¹)	CAT ($\mu\text{mol min}^{-1}$)	APX ($\mu\text{mol min}^{-1}$)	LEA prot. (rel. units)	Dehydrins (rel. units)
R5	12.3 ± 1.1d	8.4 ± 0.9d	5.1 ± 0.6d	2.3 ± 0.4e	1.8 ± 0.3e
R6	18.7 ± 1.4c	14.2 ± 1.2c	9.8 ± 0.8c	8.6 ± 1.1d	6.9 ± 0.8d
R7	24.1 ± 1.6b	19.8 ± 1.5b	15.4 ± 1.1b	22.4 ± 2.3c	18.7 ± 1.9c
R8	31.5 ± 1.9a	26.3 ± 1.8a	21.7 ± 1.4a	38.1 ± 2.8a	33.4 ± 2.6a
Post-R8	28.9 ± 1.7ab	22.7 ± 1.6ab	18.3 ± 1.3ab	35.6 ± 2.5b	31.2 ± 2.4ab

Values are means ± SE (n = 10). Relative units normalised to R5 = 1

SOD activity rose 2.6-fold from R5 to R8, while CAT and APX increased 3.1-fold and 4.3-fold, respectively (Table 2). LEA proteins reached 16.6 times their R5 level by R8. Both

protective protein classes peaked at R8 then declined slightly.

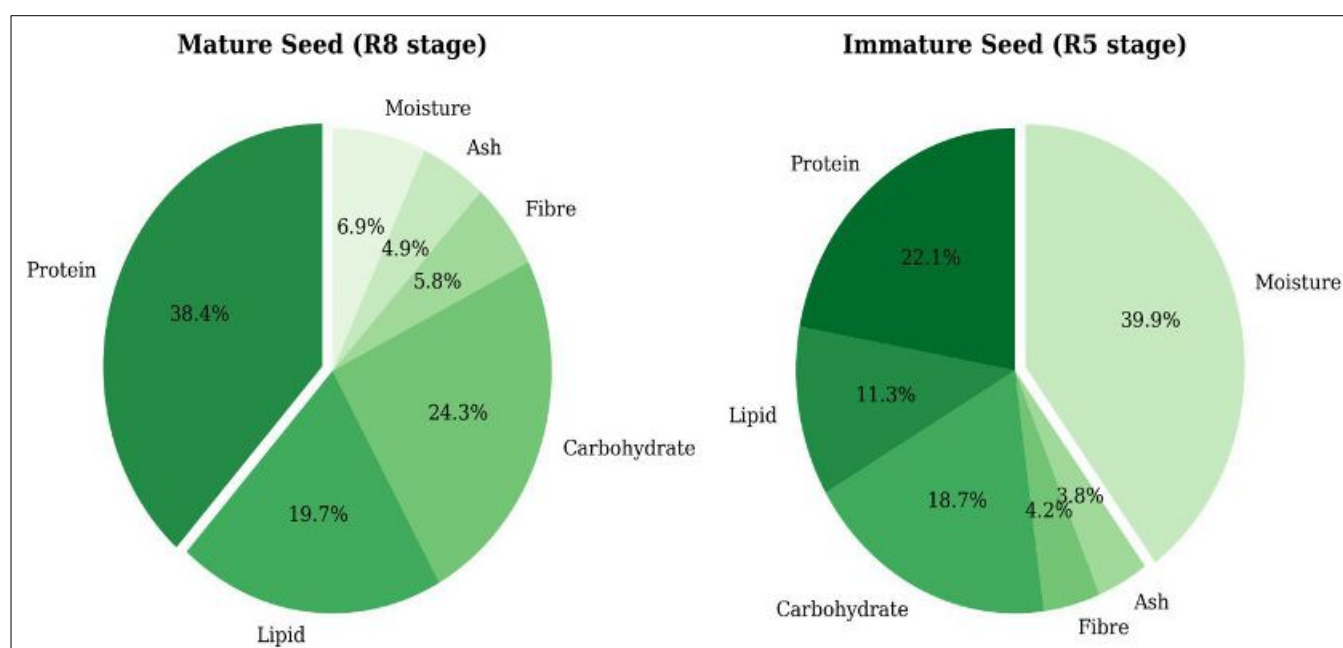


Fig 1: Proximate biochemical composition of soybean seeds at full maturity (R8, left) and early seed fill (R5, right)

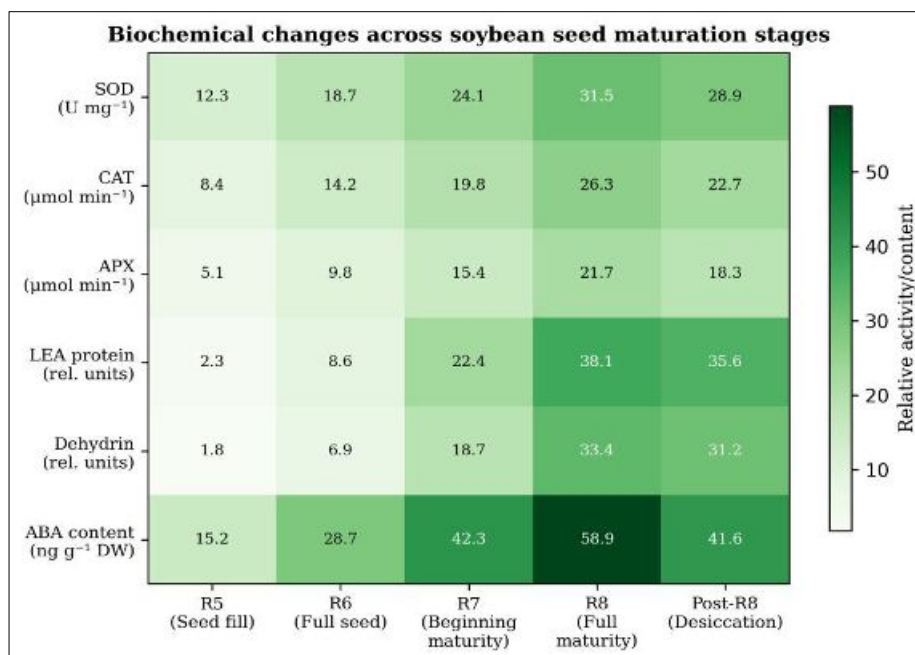


Fig 2: Heatmap of antioxidant enzymes, protective proteins, and ABA across seed maturation stages

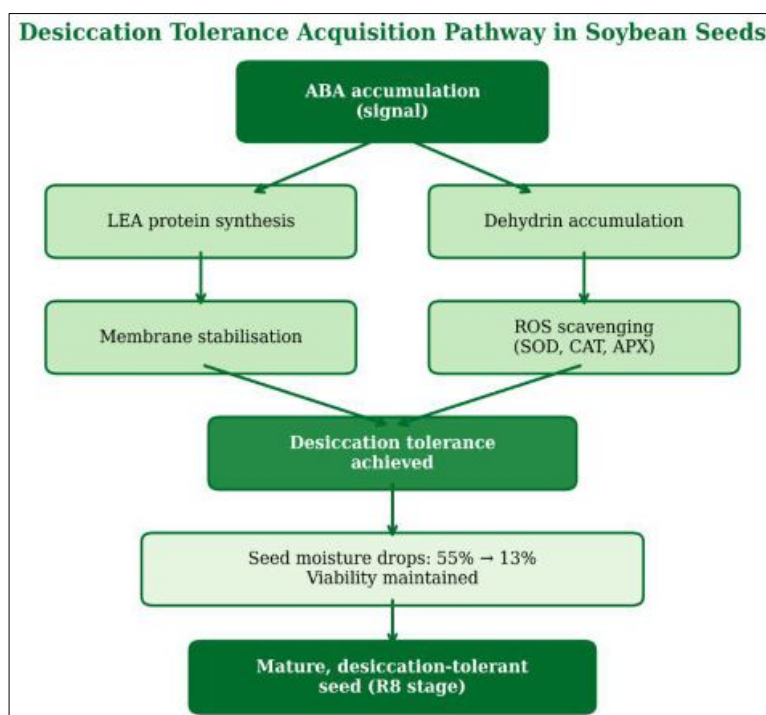


Fig 3: Desiccation tolerance acquisition pathway in soybean seeds

Discussion

The 2.6-fold increase in SOD from R5 to R8 was lower than the 3.5-fold reported for temperate soybean cultivars [8], possibly reflecting milder oxidative stress at Jimma's altitude. LEA protein accumulation was more pronounced (16.6-fold vs ~10-fold in temperate genotypes), which may be an adaptive response to rapid post-maturity drying during the Ethiopian dry season onset [9].

The decline in ABA at post-R8, while LEA proteins remained near peak levels, confirms that ABA is a transient signal rather than a structural protectant [3]. Once the protective machinery is assembled, tolerance depends on accumulated proteins and sugars rather than continued hormonal signalling [7]. From a seed technology perspective, the results reinforce the recommendation to delay harvest

until seeds reach R8 (below 13% moisture). Harvesting at R7 would result in ~30 percentage points germination loss after drying [5].

Conclusion

This research established a detailed timeline of seed maturation biochemistry in two Ethiopian soybean cultivars. The transition from desiccation-sensitive tissue at R5 to tolerant seeds at R8 was driven by ABA-mediated induction of LEA proteins, dehydrins, and antioxidant enzymes. Desiccation tolerance was essentially absent at R5 (8.3% germination) and nearly complete at R8 (92.7%). These findings carry direct implications for harvest management: farmers who harvest before R8 risk severe viability losses. Future research should examine whether foliar ABA

application during R6-R7 can accelerate tolerance acquisition.

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