South African Journal of Botany 102 (2016) 190–196

Contents lists available at ScienceDirect

South African Journal of Botany

journal homepage: www.elsevier.com/locate/sajb

The effect of temperature in moringa seed phytochemical compounds and carbohydrate mobilization

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ARTICLE INFO

Article history:
Received 28 October 2014
Received in revised form 18 June 2015
Accepted 13 July 2015
Available online 30 July 2015

Edited by AR Ndhlala

Keywords:
Abiotic stress
Antioxidants
Imbibition
Phenolic
Moringa

ABSTRACT

Temperature is one of the climatic factors that regulate seed biochemical compounds and plant physiological responses, mainly biosynthesis of carbohydrates and phytochemical compounds. This study investigated the effect of temperature on moringa seed phytochemicals' compositional changes and their utilization during seed germination. Moringa seeds were subjected to three varying temperature regimes (30/20 °C, 25/15 °C, and 20/10 °C) in germination chambers. Subsequently, the seeds were destructively sampled every 24 h interval until radicle emergence and then freeze dried for analysis. Seed performance and spectrophotometric determination of non-enzymatic and enzymatic antioxidants were carried out, while sugars were analyzed using HPLC-RID. Temperature had significant effect on speed of seed germination. Particularly, 30/20 °C accelerated seed radicle emergence with germination occurring within 48 h. Subsequently, germination was observed between 48 h and 72 h at 25/15 °C and after 72 h at 20/10 °C. Similarly, temperature especially 30/20 °C also significantly influenced the biosynthesis and accumulation of biochemical compounds in the seeds. Overall, temperature treatments of moringa seed resulted in significant differences in the rate of germination and biochemical changes, which are associated with various antioxidants and their mobilization.

1. Introduction

Moringa (*Moringa oleifera* Lam., Moringaceae) is a perennial plant known for its high antioxidant contents. It is an important food commodity with all plant parts including leaves, flowers, fruits, and immature pods possessing nutritive value (Coppin, 2008). The seeds of *M. oleifera* are known to predominantly produce phenols and different fatty acids (Tesfay et al., 2011; Lalas and Tsaknis, 2002). In plants, the phenolic antioxidants are known to exist in the free phenolic form and usually stored in the vacuole. Furthermore, free phenolics are polymerized to lignans and lignins in the plant cell wall. The produced free phenolics can be polymerized on the cell walls of developing seedlings. Apart from the antioxidants potential evident in different organs of *M. oleifera* in response to growing conditions, its adaptations and tolerance to extreme environmental conditions is favored by their unique physio-chemical and physiological characteristics.

Seeds of *M. oleifera* are reported to have multifunctional roles. The seeds in some instances are used as the best normal coagulants, which possess antimicrobial, antioxidant properties and as a result the seeds are used for purification of water (Anwar et al., 2007). The seeds also are known to have valuable nutrients for human diet and contain oil range from 49.8% to 57.25% (Osman and Abohassan, 2012; Tsaknis et al., 1998). The oil extracted from moringa seed is reported to be rich in high unsaturated fatty acid (70%) with oleic as the major component (up to 70. 52%). Also, it is found as the most stable oil since it has linolenic acid at undetectable level (Robiansyah et al., 2014). In addition, the seeds are known to possess approximately 18.9%–21.12% carbohydrate and 23.8%–33.25% protein (Al Kahtani and Abou-Arab, 1993; Oliveira et al., 1999).

Plants activate several adaptive strategies in response to abiotic environmental stresses such as temperature fluctuations, dehydration, and osmotic pressure. These adaptive mechanisms include changes in physiological and biochemical processes. Adaptation to these aforementioned stresses is associated with metabolic adjustments that lead to the accumulation of several organic solutes such as sugars, polyols, phenols, and proline (Tesfay et al., 2011).

The physiological and biochemical status of the seeds of *M. oleifera* including primary and secondary metabolites may also have huge roles for seed germination as well as post germination seedling establishment and plant development. Their adaptation to harsh conditions, which impact plant development, involves mobilization of different antioxidants. Previously, Muhl (2009) reported that 30/20 °C regime was the optimum temperature for seed germination and post-germination seedling establishment when compared to 25/15 °C and 20/10 °C. However, in the physiological and biochemical insights underlying the seed germination, seedling establishment under the optimum temperature remained speculative. It is imperative to understand the

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http://dx.doi.org/10.1016/j.sajb.2015.07.003
0254-6299/

seed germination process in relation to the antioxidant system and carbohydrate mobilization during germination at different temperature regimes (30/20 °C, 25/15 °C, and 20/10 °C). Thus, the current study aimed at evaluating the effect of these aforementioned temperatures on germination rate, antioxidant enzymes, phytochemical, and carbohydrate contents in the seeds of *M. oleifera*.

2. Materials and methods

2.1. Materials and source of seeds

All chemicals were obtained from Sigma-Aldrich®, Saarchem®, Fluka®, Separations®, or Glycoteam GmbH.

M. oleifera Lam. cultivar originally from Sudan; the seeds were generously donated by a commercial farmer and cultivated for leaf production at the Ukulinga experimental farm, Pietermaritzburg, KwaZulu-Natal. Species identification and authentication were done by a taxonomist in Bews Herbarium (NU), School of Life Sciences, University of KwaZulu-Natal, South Africa. Furthermore, a voucher of the specimen was prepared to be deposited in the Bews Herbarium, for future references.

2.2. Moringa seed germination

Moringa seeds were selected based on their size and color. Total number of 1350 seeds were sub-divided into three batches containing 450 seeds each and replicated into three (150 seeds per replication). Then for germination test, 100 seeds were arranged in moist germination paper towel and allowed to germinate in dark rooms under varying three temperature regimes (30/20 °C, 25/15 °C, 20/10 °C). Seed samples (5 seeds) were collected every 24 h for 8 weeks until radicle emergency, and the seeds freeze-dried and stored in −75 °C for further biochemical analysis. The experiment was terminated after 8 weeks for statistical analysis to determine temperature treatment effects on mobilization of seed biochemical.

Mean germination time (MGT) was also calculated according to the formulae by Ellis and Roberts (1981):

$$\mathrm{MGT} = \sum \mathrm{D}n / \sum n \quad (1)$$

where MGT is the mean germination time, n is the number of seed which were germinated on day D, and D is the number of days counted from the beginning of germination.

2.3. Determination of total antioxidant activity

Total antioxidant capacity (TAOC) was determined according to Benzie and Strain (1996) with slight modifications. These authors developed the FRAP assay which is based on the reduction of the ferric tripyridyltriazine (Fe(III)-TPTZ) complex to the ferrous tripyridyltriazine (Fe(II)-TPTZ) complex by a reductant, therefore determining the combined antioxidant capacity of antioxidant molecules present in the tissue under investigation. Aliquots of 0.1 g freeze-dried plant material were extracted with 1 N perchloric acid, vortexed and centrifuged at 12,400*g* for 10 min at 4 °C. A fresh FRAP reagent solution (300 mM sodium acetate buffer pH 3.6, 10 mM Fe(II)-TPTZ prepared in 40 mM HCl, 20 mM $FeCl_3.6H_2O$ (10:1:1)) was prepared prior to measurement. Subsequently, an aliquot of the samples (30 μL) was mixed with 900 μl FRAP reagent solution, and the absorbance was measured at 593 nm after 10 min. The total antioxidant capacity was expressed as mg $FeSO_4 \cdot 7H_2O \times$ g DW^{-1} equivalent.

2.4. Determination of free soluble prolines

Free soluble prolines were extracted according to Bates et al. (1973) with slight modifications. Briefly, approximately 0.1 g of plant material was homogenized in 10 ml of 3% aqueous sulfosalicylic acid and the homogenate filtered through Whatman no. 2 filter paper. Two milliliters of filtrate was reacted with 2 ml acid-ninhdrin and 2 ml of glacial acetic acid in a test tube for 1 h at 100 °C, and the reaction was terminated in an ice bath. The reaction mixture was extracted with 4 ml toluene, mixed vigorously with a test tube stirrer for 15–20 s. The chromophore containing toluene was pippeted out from the aqueous phase into a glass cuvette and warmed to room temperature, and the absorbance read at 520 nm using toluene for a blank. Proline standards with different dilutions were used for calibration curve. Proline concentration was determined from a standard curve.

2.5. Determination of total phenols

Phenols were determined according to Hertog et al. (1992), with slight modifications. Briefly, freeze-dried material (1 g each) was mixed with 10 ml 99.8% (v/v) methanol and vortexed for 30 s. Thereafter, the mixture was shaken overnight at room temperature to extract the free phenols. Subsequently, the mixture was centrifuged, and supernatant were filtered through Whatman no. 1 filter paper and the sample was again rinsed with 10 ml of solvent until color was no longer released. And acid hydrolysis was also used for the remaining plant residue (pellet) to efficiently release cell wall-bound phenols. Briefly, a 10 ml portion of acidified (2 M hydrochloric acid) 60% aqueous methanol was added to each sample and placed in an oven at 90 °C for 90 min exactly. Tubes were allowed to cool, and supernatants were filtered through a 0.45 μm filter and ready for analysis. The phenols concentration was determined spectrophotometrically using Folin-Ciocalteu reagent at 750 nm using gallic acid monohydrate as standard and the total phenolics concentration expressed as 'Gallic Acid Equivalents' (GAE).

2.6. Moringa carbohydrates

2.6.1. Non-structural carbohydrates

Freeze-dried material (0.10 g) was mixed with 10 ml 80% (v/v) ethanol and homogenized for 1 min. Thereafter, the mixture was incubated in an 80 °C water bath for 60 min to extract the soluble sugars. Subsequently, the mixture was kept at 4 °C overnight. After centrifugation at 12000*g* for 15 min at 4 °C, the supernatant was filtered through glass wool and taken to dryness in a vacuum concentrator. Dried samples were resuspended in 2 ml ultra-pure water, filtered through a 0.45 μm nylon filter, and sugars were analyzed according to Liu et al. (1999), using an isocratic HPLC system equipped with a refractive index detector on a Phenomenex® column (Rezex RCM–Monosaccharide). The concentration of individual sugars was determined by comparison with authentic sugar standards.

2.7. Total protein content

Total soluble proteins were extracted according to Kanellis and Kalaitzis (1992) from 1 g DW of frozen plant tissue. The extract was allowed to stand on ice for 15 min, was centrifuged at 20,000*g* for 20 min at 4 °C, and the supernatant was used for enzyme assays after being passed through Miracloth®-quick filtration material for gelatinous grindates (20–25 μm pore size; Calbiochem, San Diego, CA, USA).

The Bradford Microassay was used to determine the protein content of the samples (Bradford, 1976). Bradford dye reagent was prepared by diluting the dye concentrate with distilled water 1:4. The dye (1 ml) was added to test tubes containing 20 μl sample extract, mixed, and incubated at room temperature for 5 min. Samples were then read spectrophotometrically at 595 nm and the protein concentration determined by comparing results with a standard curve constructed using bovine serum albumin.