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Effect of germination and UV-B elicitation on chemical compositions, antioxidant activities, and phytochemical contents of underutilised Mexican blue maize seeds

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Abstract

Germination improves seed functionality due to increased phytochemicals and associated antioxidant activities. These effects are enhanced with a suitable inductor which is applied at appropriate time and dose. The aim of the present work was to evaluate the effect of germination + UV-B elicitation on the chemical compositions, antioxidant activities (AoxA), total phenolic (TPC), total anthocyanin (TA), and γ-aminobutyric acid (GABA) contents in blue maize seeds. The application of UV-B radiation (wavelengths of 280 - 311 nm) during 37.0 h was an effective elicitor. Germinated-elicited blue maize flour (GEBMF) had higher proteins (+29.1%), dietary fibres (+22.0%), and AoxA (ABTS: +133.9%; DPPH: +173.4%) than unprocessed blue maize flour (UBMF). The increase in AoxA was closely related to the observed increase in TPC (+587.2%), TA (+29.9%), and GABA (+199.9%). Therefore, GEBMF could be used as a source of proteins, dietary fibres, and natural antioxidants in the formulation of new functional foods and beverages. These results could also contribute to the use and conservation of blue maize, an underutilised cereal.

Introduction

Mexico is considered as the centre of origin and domestication of maize (*Zea mays* L.); there are approximately 59 different maize landraces throughout the country which have been obtained by selection and reproduction of the best grains (Rivera-Castro *et al.,* 2020). At least 13 of these landraces have been identified in the state of Sinaloa, where Tabloncillo and Elotero de Sinaloa (blue maize) are widely distributed (Pineda-Hidalgo *et al.,* 2013). Blue maize is mainly consumed as tortillas and tortilla chips. Products derived from blue maize have received increased attention due to their potential health benefits (antioxidant, antimutagenic, anti-inflammatory, hypoglycaemic, hypercholesterolaemic, anti-atherosclerotic, anti-obesogenic, antiaging, and anticancer activities) mainly due to the presence of phenolic compounds (phenolic acids and anthocyanins) (Urías-Lugo *et al.,* 2015; Bello-Pérez *et al.,* 2016; Chavarín-Martínez *et al.,* 2019a). However, despite the nutraceutical properties of blue maize and other maize landraces, the use of these crop commodities is very low; thus, it is necessary to develop some technological alternatives to improve the use and conservation of these underutilised seeds.

Grain germination is a simple and economic strategy widely used to naturally improve the physicochemical properties, nutritional qualities, and nutraceutical contents, while also reducing the anti-nutrient contents (phytic acid, tannins, and trypsin inhibitors). During germination, some hydrolytic enzymes degrade the main seed storage molecules (carbohydrates, proteins, and lipids), thus causing a readjustment in the content and type of these molecules, and at the same time, the development of flavours, textures, and aromas. This bioprocess also activates metabolic pathways responsible for the synthesis of phytochemicals associated with antioxidant activities and nutraceutical properties, including those involved in the protection and reducing risk of developing some chronic diseases such as cancers, diabetes, hypertension, neurological disorders, and

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cardiovascular diseases (Chavarín-Martínez *et al.,* 2019b).

Recent studies indicated that germinated cereal grains show a higher content of phytochemical compounds such as γ-aminobutyric acid (GABA), total phenolics, total anthocyanins, carotenoids, and antioxidant activities than those of ungerminated seeds (Chalorcharoenying *et al.,* 2017; Gong *et al.,* 2018; Chavarín-Martínez *et al.,* 2019b). Germination at optimal conditions has also been reported as an effective strategy to increase proteins, total phenolics, total anthocyanins, and antioxidant activities of blue maize seeds (Chavarín-Martínez et al., 2019a). The stage of germination in pigmented maize is a source of variations for antioxidant activities and phytochemical compounds (carotenoids, GABA, phenolics, and anthocyanins). Germination could be at sprouting (seed germinated in water, and harvested before the development of true leaves, which is intended to be eaten whole, including the seed) or seedling (obtained at fully expanded cotyledon stage or first true leaf stage; the product is consumed including stem, cotyledons, and first true leaves). Germinated seedlings have shown the highest antioxidant and phytochemical values (Chalorcharoenying *et al.,* 2017; Benincasa *et al.,* 2019; Chavarín-Martínez *et al.,* 2019a). Cereal seedlings can be consumed in the form of ready-to-eat sprouts or further processed, for example, dried, toasted, and extruded. The reported uses of germinated cereals are as fresh juice, tablets, capsules, liquid concentrates, functional beverages (obtained by lactic acid fermentation of mixture based on germinated grains and flour), as well as flours for supplementation of bakery and confectionery products, tortillas, noodles, pastas, laddus, and porridges (Benincasa *et al.,* 2019).

The application of physical or chemical stresses during germination, known as elicitation, is useful as a strategy to increase the phytochemical contents in different sprouts. The elicitation process could increase the activity of enzymes involved in the synthesis of low-molecular antioxidants like tyrosine/phenylalanine ammonia-lyase, chalcone synthase, known as crucial enzymes in the phenylpropanoid pathway (phenolic synthesis) (Świeca, 2016). The application of elicitors [chemical solutions, hypoxic conditions, thermal stress, continuous light radiation (including various types of light like red and blue), and UV radiation] has been used during the different phases of soaking and germination to increase the accumulation of bioactive compounds and antioxidant activities (Baenas *et al.,* 2014). UV radiation in crops can generate a reduction of growth rate and primary productivity, and changes in ultrastructure by altering the levels of chlorophylls a and b, and total chlorophylls. UV radiation often is divided into UV-A (320 - 390 nm), UV-B (280 - 320 nm), and UV-C (200 - 280 nm). The UV-A and UV-B radiations are related to several photomorphogenic reactions in crops, for instance, the synthesis of flavonoids, compounds mainly located in the outer layers of seeds and plants acting as UV-protecting agents by absorption of sunlight UV-A and UV-B (de Almeida *et al.,* 2013; Shaukat, *et al.,* 2013).

Different researches have established that UV-B can induce stress, physiological and photomorphogenic responses, thus affecting growth, development, biomass accumulation, yield, and the plants' metabolism. The inhibition of stem growth affects the shoot morphology, thus leading to increased leaf thickness, alterations in the cuticle, and increased production of UV-B protective pigments in different crops. During UV-B elicitation, a protective mechanism is to increase the levels of chlorophylls, phenolics, flavonoids, anthocyanins, and other compounds that protect the sensitive tissues from UV-B radiations (Eguchi and Sato, 2009; Falcone-Ferreyra *et al.,* 2012; Tsurunaga *et al.,* 2013).

The objective of the present work was therefore to study the effects of germination + UV-B elicitation on the chemical compositions, antioxidant activities (AoxA), total phenolic (TPC), total anthocyanin (TA), and γ-aminobutyric acid (GABA) contents in blue maize seeds.

Materials and methods

Materials

The pigmented blue maize seeds (Elotero de Sinaloa) were acquired from the Genetic Improvement Program, Agronomy Faculty, Autonomous University of Sinaloa, Culiacán, Sinaloa, Mexico. The seeds were harvested in the summer of 2018, having the following characteristics: dimensions of 10.25 mm length × 10.01 mm width × 5.22 mm thickness (average of 100 seeds randomly selected); and seed weight value of 314.03 g (average of 1,000 seeds randomly selected) and 79.35 kg/100 L (test weight). The average germination was 93%. All the measurements were carried out in triplicate. The seeds were cleaned, packed in plastic bags, and stored at 4 - 8°C in tightly sealed containers until further use. The difference in storage time was less than four months between the different trials. At the time of the

experiments, they were randomly assigned to the experimental units (blue maize seeds). The UV-B elicitation was conducted using linear fluorescent bulbs (PT2162 5.0/T8 30" 75 cm 25 W) UV-B wavelength emission of 280 - 311 nm.

Methods

Production of germinated-elicited blue maize flours (GEBMF)

Germination was carried out as reported by Chavarín-Martínez *et al.* (2019a), with some modifications. A portion of 200 g of blue maize seeds were soaked in 1,000 mL of 0.1% sodium hypochlorite for 10 min. Then, the seeds were washed with distilled water, and soaked (25°C/12 h) with 1,000 mL of distilled water. The hydrated seeds were placed in germination trays on wet laboratory paper, and into the germination chamber at 26.9°C. A relative humidity (RH) of 80 - 90% within the chamber was maintained using trays with water. Following 96 h of germination, the elicitation process was carried out by exposing to UV-B light (wavelengths of 280 - 311 nm) in the interval of 0 - 111 h (Table 1; 10 treatments). After the elicitation process, the germination process continued until reaching 207.7 h (seedling stage). In all cases, the seeds were germinated under 50% light/50% darkness periods [light source: fluorescent tubes (white light, 16 W/2,700 K, Tecno Lite, China)]. The resulting germinated-elicited blue maize seeds were dried (50°C/8 h), tempered (25°C), and ground (80-US mesh = 0.180 mm) to obtain germinated-elicited blue maize flours (GEBMF). The GEBMF was packed and kept at 4°C in tightly sealed containers until further analysis.

Extraction of free and bound phenolic compounds

Free and bound phenolic compounds were extracted using 80% chilled ethanol and ethyl acetate as solvents, respectively (Perales-Sánchez *et al.* 2014). All measurements were performed in quadruplicate.

Antioxidant activities (AoxA): ABTS, DPPH

The hydrophilic AoxA in free and bound phenolic extracts was determined by ABTS and DPPH assays as reported by Brand-Williams *et al.* (1995) and Re *et al.* (1999), respectively, and expressed as mmol of Trolox equivalents (TE)/100 g sample (DW). All measurements were performed in triplicate.

Total phenolic contents

The total phenolic contents of free and bound extracts were determined according to

Table 1. Effect of UV-B irradiation duration on the antioxidant activity[a] and total phenolic content[a] of germinated-elicited blue maize flours (GEBMF).

Assay[b]	UV-B irradiation duration[c]	Antioxidant activity[d]	Total phenolic content[e]
1	0.0	23.39 ± 1.58^{DE}	895.6 ± 42.2^{BCD}
2	12.3	24.80 ± 0.74^{CD}	1145.1 ± 24.3^{AB}
3	24.7	20.01 ± 1.42^{F}	873.1 ± 133.1^{BCD}
4	37.0	28.17 ± 0.95^{A}	1348.2 ± 148.0^{A}
5	49.3	24.29 ± 1.55^{CD}	732.8 ± 41.7^{CD}
6	61.7	23.35 ± 0.27^{DE}	872.3 ± 115.3^{BCD}
7	74.0	25.87 ± 0.12^{BC}	957.0 ± 74.4^{BC}
8	86.3	21.45 ± 1.16^{EF}	660.9 ± 21.6^{D}
9	98.7	25.59 ± 1.60^{C}	913.4 ± 150.5^{BCD}
10	111.0	27.81 ± 1.12^{AB}	1231.0 ± 352.7^{A}

[a]Values are mean ± standard deviation. Means with different uppercase superscripts in the same column are significantly different (Duncan, $p \leq 0.05$); [b] Does not correspond to the order of processing; [c] hours (h); [d] (ABTS assay) mmol Trolox equivalents (TE)/100 g (DW); and [e] mg gallic acid equivalents (GAE)/100 g sample (DW).

Singleton *et al.* (1999), and expressed as mg of gallic acid equivalents (GAE)/100 g sample (DW). All measurements were performed in triplicate.

Experimental design, statistical analysis, and selection of duration of the UV-B elicitation process

The experimental design was completely randomised design of one factor (time of UV-B irradiation duration) and ten levels (0.0, 12.3, 24.7, 37.0, 49.3, 61.7, 74.0, 86.3, 98.7, and 111.0 h) to a total of ten treatments; each treatment was performed in triplicate. The experimental results of AoxA and TPC was analysed by one-way ANOVA, and means were compared by Duncan's multiple range test with a significance level of 5%. The software Statgraphics Plus was used for the data analysis, and selected the elicitation process's duration with UV-B radiation when analysing the information provided by the statistical analysis of the experimental data.

Chemical compositions, soluble, and insoluble dietary fibres

The following methods of the Association of Official Analytical Chemists (AOAC, 2012) were referred to evaluate the proximate composition: moisture (method 925.09B), drying at 130°C; lipids (method 920.39C), defatting in a Soxhlet apparatus with petroleum ether; minerals (method 925.21), incineration at 550°C; and protein (method 960.52), micro-Kjeldahl (N×6.25). Soluble and insoluble dietary fibres (SDF, IDF) were evaluated using the enzymatic-gravimetric method for total dietary fibres (TDF) (method 985.29), using the TDF assay kit from Sigma-Aldrich (TDF 100 A) (AOAC, 2012). Carbohydrate content was estimated by difference.

Anthocyanin contents

Extractions of anthocyanins were performed using acidified methanol solution. The total anthocyanin contents were determined using the methodology reported by Abdel-Aal and Hucl (1999), and expressed as mg of cyanidin 3-glucoside equivalent (CGE)/100 g sample (DW). All measurements were performed in triplicate.

γ-aminobutyric acid contents

The GABA contents were determined using the methodology reported by Wathararparpaiboon *et al.* (2010), and expressed as mg of GABA equivalent/100 g sample (DW). All measurements were performed in triplicate.

Experimental design and statistical analysis for the chemical compositions, antioxidant activities, total phenolic, total anthocyanin, and γ-aminobutyric acid contents of blue maize flours

The experimental design was completely randomised design of one factor (the type of flour) and three levels (UBMF = unprocessed blue maize flour, GBMF = germinated blue maize flour, and GEBMF = germinated elicited blue maize flour). The experimental results of chemical compositions, antioxidant activities, total phenolic, total anthocyanin, and γ-aminobutyric acid contents of the blue maize flours were analysed by one-way ANOVA. The means were compared by Duncan's multiple range test with a significance level of 5%. The software Statgraphics Plus was used for the data analysis.

Results and discussion

Selection of duration for the UV-B elicitation process

Table 1 shows the means of ten different UV-B radiation durations and experimental values of the AoxA and TPC from the germinated-elicited blue maize flours (GEBMF). The AoxA and TPC in GEBMF showed an oscillatory behaviour (Figure 1). The values of AoxA and TPC varied from 20.01 to 28.17 mmol TE/100 g sample (DW), and from 660.9 to 1348.2 mg GAE/100 g sample (DW), respectively. The experimental values came from different elicitation conditions (different UV-B radiation durations). Data analysis showed that 87 and 75% of the total variation of the AoxA and TPC was explained by the UV-B radiation duration factor, and the rest of the variation in the data was due to experimental error (13 and 25% for AoxA and TPC, respectively). This oscillatory behaviour could be due to the generation and utilisation of phytochemicals by the plant with potential photoprotective ability. When blue maize was subjected to UV-B radiation, protection mechanisms might be activated against UV-B rays' damage, but the use of these phytochemicals by the plant might decrease their content during some assays. The oscillatory behaviour could be supported by the research of Shaukat *et al.* (2013) which reported that both the time of UV-B exposure and the time of germination affected the accumulation of phytochemicals in mash-bean (*Vigna mungo* (L.) Hepper.) seeds.

Elicitation by UV-B radiation is an effective way to enhance AoxA activity, and TPC and anthocyanin syntheses in several cereal seeds. These effects depend on different factors such as the nature

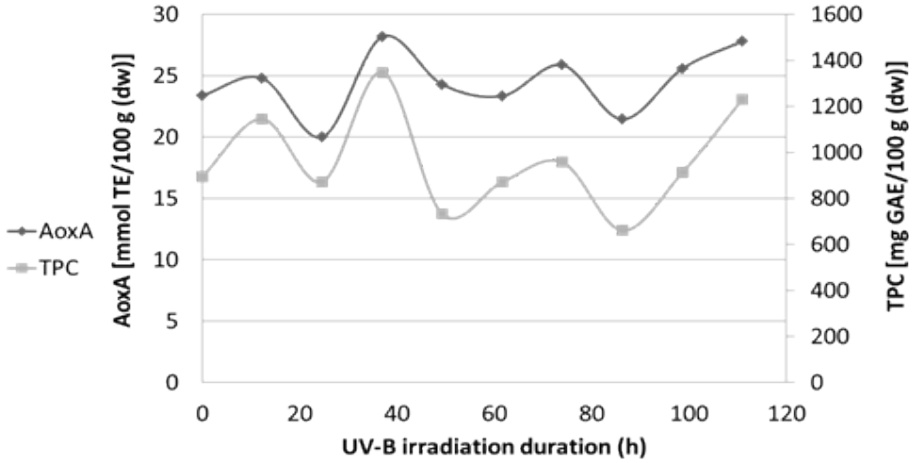

Figure 1. Changes in AAox and TPC experimental values at different values of UV-B irradiation durations.

of the elicitor, dose, and time of exposure, which affect the intensity of the plant response. Elicitors can stimulate different kinds of secondary metabolites, and change their concentration depending mainly on genetic (species and cultivate), physiological (age, organ, and maturation), and agronomic (stress, photoperiod, and fertilisation) factors. Stress is one factor that produces significant alterations in the content and composition of bioactive secondary metabolites in crops. The concentration of elicitor and the interval between treatment and harvest induce different characteristic responses of plant species, thus making it necessary to find the appropriate and effective dose and application time (Falcone-Ferreyra *et al.,* 2010; 2012; Shaukat *et al.,* 2013; Tsurunaga *et al.,* 2013; Baenas *et al.,* 2014).

The highest values of AoxA [28.17 mmol TE/100 g sample (DW)] and TPC [1348.2 mg GAE/100 g sample (DW)] were obtained with 37.0 h of UV-B radiation (wavelengths of 280 - 311 nm). Therefore, this duration of elicitation was selected for further analyses.

Chemical compositions

Table 2 shows the chemical compositions, antioxidant activities, and the contents of phenolics, anthocyanins, and *γ*-aminobutyric acid in blue maize flours. UV-B radiation did not affect the chemical compositions, having significant ($p < 0.05$) differences only in the dietary fibre contents (soluble and total) (Table 2). Therefore, the differences in chemical compositions between GEBMF and UBMF (unprocessed blue maize flours) could be attributed to the germination bioprocess.

GEBMF had higher contents of proteins (11.72 vs 9.08%), minerals (1.83 vs 1.59%), and total dietary fibres (16.26 vs 13.33%), as well as lower contents of lipids (4.32 vs 4.59%) and carbohydrates (65.15 vs 71.40%) than UBMF. The protein and mineral contents increased by +29% and +15%, respectively, in GEBMF as compared to UBMF. This corresponds with previous studies which reported an increase between 4 and 40% during maize germination (Kavitha and Parimalavalli, 2014; Gong *et al.,* 2018; Chavarín-Martínez *et al.,* 2019a). The increase in protein and mineral contents can be attributed mainly to the loss of dry weight due to the oxidation of carbohydrates during respiration (Perales-Sánchez *et al.,* 2014). It could also be associated with an increase in the activity of enzymes related to protein synthesis during germination, or the release of minerals due to a decrease in the contents of phytates and tannins by enzymatic activity (Baenas *et al.,* 2014).

The lipid content of UBMF was significantly ($p < 0.05$) higher than that of GEBMF. These results are similar to those reported by other researchers (Kavitha and Parimalavalli, 2014; Chavarín-Martínez *et al.,* 2019b) who reported that sprouted maize flour contained less fat than unprocessed maize flour. They attributed this result to the fact that lipids are used as a source of carbon and energy for seed sprouting and seedling during the germination process. Lipid catabolism provides energy and carbon sources needed for the biochemical and physicochemical modifications taking place during seedling growth. Several researchers (Kubicka *et al.,* 2011; Makinen and Arendt, 2012) reported a 1.2- to 2.3-fold increase in the activity of lipase and lipoxygenase during the sprouting of cereal grains due to their *de novo* synthesis in the aleurone and scutellum. Increasing the sprouting temperatures leads to a higher lipid

Table 2. Chemical compositions, antioxidant activities, and contents of phenolic compounds, anthocyanin, and GABA in blue maize flours.

Property	UBMF	GBMF	GEBMF
Chemical composition (%, DW)			
Protein	9.08 ± 0.23^{B}	11.15 ± 0.31^{A}	11.72 ± 0.33^{A}
Lipid	4.59 ± 0.15^{A}	4.21 ± 0.05^{B}	4.32 ± 0.04^{B}
Mineral	1.59 ± 0.04^{B}	1.72 ± 0.02^{AB}	1.83 ± 0.13^{A}
Dietary fibre			
Soluble	1.96 ± 0.07^{A}	0.49 ± 0.02^{C}	0.80 ± 0.04^{B}
Insoluble	11.38 ± 0.20^{C}	24.26 ± 0.63^{A}	16.18 ± 0.50^{B}
Total	13.34 ± 0.33^{C}	24.75 ± 0.52^{A}	16.98 ± 0.52^{B}
Carbohydrate	71.40 ± 1.41^{A}	58.17 ± 1.20^{C}	65.15 ± 2.76^{B}
Antioxidant activity[a]			
ABTS			
Free phenolic	0.94 ± 0.19^{C}	4.39 ± 0.17^{B}	5.81 ± 0.17^{A}
Bound phenolic	11.48 ± 1.81^{B}	19.58 ± 1.88^{A}	23.25 ± 3.87^{A}
Total phenolic	12.42 ± 1.66^{C}	23.97 ± 1.96^{B}	29.06 ± 3.15^{A}
DPPH			
Free phenolic	0.41 ± 0.02^{C}	5.05 ± 0.14^{B}	6.20 ± 0.27^{A}
Bound phenolic	3.68 ± 0.43^{B}	4.75 ± 0.09^{A}	4.97 ± 0.33^{A}
Total phenolic	4.09 ± 0.13^{C}	9.80 ± 0.99^{B}	11.17 ± 0.13^{A}
Phenolic compound[b]			
Free phenolic	42.03 ± 8.22^{C}	342.54 ± 11.34^{B}	524.85 ± 25.34^{A}
Bound phenolic	199.76 ± 25.34^{C}	609.88 ± 62.33^{B}	1136.66 ± 153.87^{A}
Total phenolic	241.79 ± 22.06^{C}	952.42 ± 88.81^{B}	1661.51 ± 112.52^{A}
Total anthocyanin content[c]	29.85 ± 0.10^{C}	32.53 ± 2.18^{B}	36.14 ± 0.72^{A}
GABA content[d]	9.80 ± 3.26^{C}	21.25 ± 0.89^{B}	29.38 ± 3.90^{A}

Values are mean ± standard deviation. Means with different lowercase superscripts in the same row are significantly different (Duncan, $p \leq 0.05$); [a] mmol Trolox equivalents (TE)/100 g (DW); [b] mg gallic acid equivalents (GAE)/100 g sample (DW); [c] mg of cyanidin 3-glucoside equivalent (C3GE)/100 g sample (DW); and [d] mg of γ-aminobutyric acid (GABA)/100 g sample (DW). UBMF = unprocessed blue maize flour; GBMF = germinated blue maize flour; and GEBMF = germinated-elicited blue maize flour.

breakdown. A decrease in total lipid content of 18 to 28% was observed in millet sprouted at 32°C for two days (Inyang and Zakari, 2008), brown rice grown at 25 to 30°C for one to five days (Watanabe *et al.,* 2004; Cáceres *et al.,* 2014), and wheat sprouted at 30°C for two days (van Hung *et al.,* 2011). Lipase catalyses the degradation of triglycerides to glycerol and free fatty acids, which are mainly converted to sucrose that is sent to the scutellum for use by the rootlet and shoot (Kubicka *et al.,* 2011).

The germination process increased ($p < 0.05$) the total and insoluble dietary fibres, while soluble dietary fibres decreased in GEBMF and GBMF as compared to UBMF. Some researchers (Inyang and Zakari, 2008; Cáceres *et al.,* 2014; Gong *et al.,* 2018) reported similar trends in maize and other cereals, which were attributed to increased metabolic fluxes, like the production of cellulose, hemicellulose, and pectin polysaccharides (quantified as total and insoluble fibres). Also, this

increase in fibre could be due to some modifications that occur in the structure of cell wall polysaccharides of the seeds during germination, possibly by disrupting the protein-carbohydrate interaction and affecting the intactness of tissue histology (Tsurunaga *et al.,* 2013; Perales-Sánchez *et al.,* 2014).

The insoluble and total fibre contents of GEBMF were significantly ($p < 0.05$) lower than those in GBMF, while soluble fibre was higher in GEBMF than in GBMF. This could be due to the fact that UV-B elicitation generated sprouts affected visibility in leaf morphology/development (leaf index and leaf area) and lower plant biomass [root and shoot weights (dry and fresh)] when compared with GBMF (Figure 2). A similar behaviour has been previously reported by other researchers (de Almeida *et al.,* 2013; Shaukat *et al.,* 2013), which could be attributed to the impact of the secondary metabolic pathways during UV-B stress on the plant physiology, thus suppressing the growth of the cotyledons and the first leaf.

Antioxidant activities

Germinated elicited blue maize flour (GEBMF) and germinated blue maize flour (GBMF) had higher antioxidant activity (ABTS) than unprocessed blue maize flour (UBMF), with values of 29.07, 23.97, and 12.43 mmol TE/100 g sample, DW, respectively (Table 2). UV-B elicitation enhanced significantly ($p < 0.05$) the antioxidant activity in GEBMF as compared to GBMF and UBMF (Table 2). A similar result was observed for the antioxidant activity by the DPPH assay. These results emulate those obtained for TPC, thus suggesting that phenolic compounds have a significant contribution to the AoxA of GEBMF and GBMF. This statement is supported by the highly significant positive correlation ($p = 0.01$, $r = 0.77$) found between TPC content and ABTS values. These results are consistent with those of other authors, thus indicating that elicitors increased the phenolic content, and consequently, the antioxidant activity of sprouts (Falcone-Ferreyra *et al.,* 2010; 2012; Baenas *et al.,* 2014).

In general, germination increases the antioxidant activity of cereals. Various researchers have shown a 1.2- to 2.9-fold increase in antioxidant activity in wheat (Hithamani and Srinivasan, 2014), brown rice (Cáceres *et al.,* 2014; Cornejo *et al.,* 2015), and oats (Xu *et al.,* 2009) when sprouted at 15 to 28°C for two to five days. The higher antioxidant activity in sprouted grains is mainly attributed to the accumulation of polyphenols, which play a role in the protection of plants against environmental stresses. Their antioxidant activities are associated with scavenging free radicals, breaking radical chain reactions, and chelate metals. The main polyphenols in cereal grains are *p*-hydroxybenzoic, ferulic, sinapic, vanillic, *p*-coumaric acids, and in oats are avenanthramides. Generally, 60 to 90% of the polyphenols in cereals occur in a bound form (Xu *et al.,* 2009; Hithamani and Srinivasan, 2014).

Phenolic contents

As shown in Table 2, the GEBMF, GBMF, and UBMF had TPC of 1661.52, 952.41, and 241.79 mg gallic acid equivalents (GAE)/100 g sample (DW), respectively. Both bioprocesses (germination and germination + UV-B elicitation) increased the TPC in blue maize seed. The germination + UV-B elicitation increased ($p < 0.05$) the free (+1148%), bound (+469%), and total (+587%) phenolic contents in blue maize seeds. de Almeida *et al.* (2013) reported increases of phenolic compounds up to 66.24% in *O. sativa* exposed to UV-B radiation.

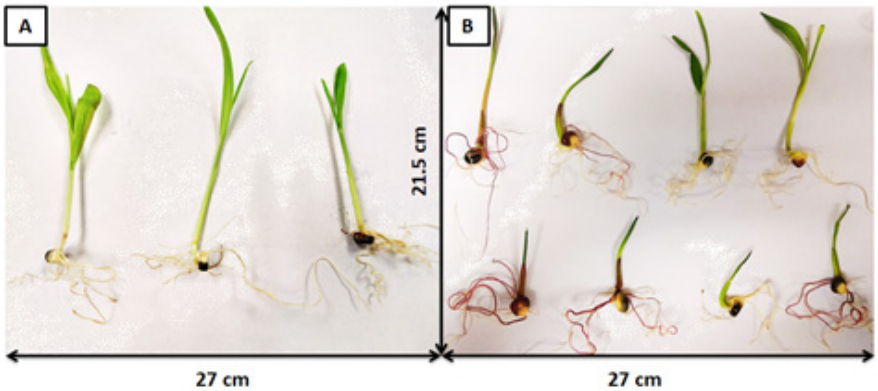

Figure 2. Effect of the UV-B elicitation on the appearance of maize sprouts (207.7 h of germination under 50% light / 50% darkness periods). (A) Control sprouts without UV-B radiation, and (B) blue maize sprouts under selected UV-B elicitation conditions (37.0 h of UV-B radiation duration).

The accumulation of phenols resulting from the plants' exposure to UV-B radiation has also been reported by other authors (Mark and Tevini, 1996; Shaukat *et al.,* 2013). Phenolic compounds are active antioxidant compounds advocated by cellular oxidative conditions as a strategy against reactive oxygen species (ROS). UV-B radiation increases the content of phenolic compounds, thus indicating that it is a photoprotective mechanism against UV-B damage (de Almeida *et al.,* 2013).

Phenylalanine ammonia-lyase and tyrosine ammonia-lyase (PAL and TAL, respectively) activities increased in response to UV-B stress. These enzymes convert amino acids to secondary metabolites such as phenolic acids including lignins, flavonoids, and phytoalexins. Secondary metabolites are the plant defence mechanism to protect them from pathogens, insects, and other sources of physical stresses such as UV-B radiation. Some of them have the capacity to absorb UV-radiation, thus preventing severe leaf damage (de Almeida *et al.,* 2013; Shaukat *et al.,* 2013; Baenas *et al.,* 2014). The increases in TPC could contribute to the synthesis of UV-B protecting agents such as ferulic acid, which could double the photoprotection to solar-simulated irradiation, thus conferring strong UV absorptive ability to its chemical structure (Ruhland *et al.,* 2007).

The protective role of phenolics in response to UV-B radiation may be due to the structural stabilisation of cell wall through condensation-polymerisation of phenols and quinines. They can provide a photoprotective mechanism against the potential damage of UV-B by absorbing and quenching these specific wavelengths, and enhance the oxidative damage caused by increasing ROS (Lin *et al.,* 2005).

Anthocyanin contents

GEBMF and GBMF had higher anthocyanin contents than UBMF with values of 36.14, 35.53, and 29.85 mg of cyanidin 3-glucoside equivalent (C3GE)/100 g sample (DW), respectively (Table 2). Germination + UV-B elicitation caused a significant increase (+21%) in the total anthocyanin contents of blue maize seeds. The increase in maize anthocyanins in response to UV-B radiation corroborates previous findings (Tsurunaga *et al.,* 2013) where the levels of anthocyanins and flavones increased by several folds as compared to the controls (maize germinated in the dark). During germination + UV-B elicitation, the activity of two of the main enzymes (PAL and TAL) involved in the anthocyanin biosynthesis pathway increased, whereas UV-B radiation exposure enhanced the TAL activity (Shaukat *et al.,* 2013).

Furthermore, the systematic accumulation of UV-B absorbing pigments such as flavonoids and anthocyanins provide one of the primary mechanisms, through which, plants mitigate UV-B radiation effects. These compounds are well known for their capacity as protective pigments. Anthocyanins play an essential role in radiation absorption when the plants are exposed to UV-B radiation (Falcone-Ferreyra *et al.,* 2010; Shaukat *et al.,* 2013).

γ-aminobutyric acid contents

γ-aminobutyric acid (GABA), an essential non-protein amino acid, functions as the predominant inhibitory neurotransmitter in the central nervous system. It is also effective at decreasing blood pressure and treating epilepsy. It is formed by transamination of *α*-ketoglutarate to glutamic acid, which is then decarboxylated by a glutamic acid decarboxylase to GABA (Ramesh *et al.,* 2017).

As shown in Table 2, the GEBMF, GBMF, and UBMF had GABA contents of 29.38, 21.25, and 9.80 mg GABA/100 g sample (DW), respectively. GABA content in GEBMF was three times higher than that of UBMF (Table 2). GABA shunt has been an essential mechanism preventing the accumulation of ROS intermediates and cell death under UV-B stress conditions. Therefore, the accumulation of GABA in UV-B elicited maize sprouts may help prevent oxidative stress damage (Ramesh *et al.,* 2017).

The GABA content of blue corn flours is similar to that reported for other unprocessed and germinated cereal seeds such as barley and wheat [less than 2 mg/100 g (DW)] (Ohm *et al.,* 2016), regular brown rice [3 to 7 mg/100 g (DW)] (Cornejo *et al.,* 2015; Chen *et al.,* 2016), and also sprouted barley and wheat [7 to 25 mg/100 g (DW)] (Chung *et al.,* 2009; Ohm *et al.,* 2016).

Conclusion

Germination-elicitation improves the seeds' functionality due to the increase in bioactive compounds and associated antioxidant activities. It is also a technologically accessible bioprocess which requires low economic investment; the necessary infrastructure is minimal, can carry out at any time, and does not generate effluents. Germinated-elicited blue maize flour (GEBMF) had higher content of proteins, dietary fibres, and antioxidant activities

than unprocessed blue maize flour (UBMF). The increase in antioxidant activities was associated with the increase in total phenolic contents, anthocyanins, and γ-aminobutyric acid, thus suggesting that these compounds are the main antioxidants. Therefore, GEBMF could be used as a source of proteins, dietary fibres, and natural antioxidants in the formulation of new functional foods and beverages. These results could also contribute to the use and conservation of blue maize, an underutilised cereal.

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