Micronutrient seed priming improves maize (Zea mays) early seedling growth in a micronutrient deficient soil

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ABSTRACT

Micronutrient deficiency is a major constraint to crop productivity in South Africa. Agronomic interventions such as nutrient seed priming (NSP) could significantly improve stand establishment in micronutrient deficient soils. However, the effectiveness of the technique depends on the efficacy of the priming procedures. Laboratory and glasshouse studies were carried out to determine the effects of NSP concentration of Zn, B and Mo and priming duration on maize germination and seedling emergence and early growth in micronutrient deficient soils. Five concentrations: 0.01%, 0.05%, 0.1%, 0.5% and 0% (control) and three priming durations: 24 h, 12 h and 8 h were used for the laboratory experiment whilst the 0.5% concentration and 8 h duration were excluded in the glasshouse experiment. Seed priming duration and concentration levels and their interactions had significant (P < 0.05) effects on germination percentage (GP), germination rate (GR), the coefficient of velocity of germination (CVG), days to germination (DG) and mean germination time (MGT). These parameters were improved by priming at low concentration of the micronutrients for longer. Similarly, under glasshouse conditions, NSP at the lowest concentration but for the longest duration resulted in up to 50% earlier seedling emergence over the control. Priming with 0.01% Bo reduced the number of days to seedling emergence by 94%, increased fresh and dry seedling mass and chlorophyll content index by 29%, 47% and 58% respectively relative to the control. The earlier seedling emergence could have also contributed to superior fresh and dry seedling mass as well as both higher shoot and root mass over the control. Moreover, NSP enhanced chlorophyll content index, which could have ultimately led to better shoot growth. This suggests that with optimum micronutrients concentration levels and appropriate priming duration NSP can improve germination and seedling growth and hence maximization of the yield parameters.

1. Introduction

Maize yield largely depends on the optimum establishment of vigorous seedlings, which in turn depends on the adequate supply of essential plant nutrients (Finch-Savage and Bassel, 2016; Lutts et al., 2016). Vigorous seedlings that have well developed root systems are better able to withstand micronutrient deficient conditions as well as other biotic and abiotic stresses (Harris, 2006; Dimkpa and Bindraban, 2016). However, most resource-poor farmers in South Africa grow maize on marginal lands and infertile soils, which have an inherent low concentration of not only essential macro-nutrients but micro-nutrients as well (Barnard and du Preez, 2004; Mandiringana et al., 2005). Consequently, there is both poor establishment and early growth and hence low yields, which ultimately threatens food security (Singh et al. 2014).

Whilst applications of fertilisers could assist resource-poor farmers to improve establishment of vigorous maize stands (FSSA, 2007a), most smallholder farmers fail to apply sufficient fertilizers to address all macro-and micro nutrient deficiencies in maize due to high input cost and lack of technical knowledge (Harris et al., 2007; Moswetsi et al., 2017).

Whereas a lot of soil fertility management technologies have been devised to optimise the establishment of vigorous seedlings, adoption by resource-poor farmers remains low because they are either too expensive for the resource poor farmers, or simply inappropriate (von Loeper et al., 2016). The introduction of low-cost and low-risk agronomic interventions that can easily be adopted by resource-poor farmers could significantly improve stand establishment and yield (Harris et al. 2005). One such technology, which can boost yields for poor resource farmers
on soils of low fertility is nutrient seed priming (NSP). Essentially, NSP is a technique in which, seeds are soaked in nutrient solution containing essential micronutrients such as zinc, boron, molybdenum and macro-nutrients such as phosphorous, instead of the traditional pure water (Farooq et al. 2019). The aim of NSP is to increase seed nutrient contents along with the priming effect to improve seed quality for better crop establishment (Imran et al., 2013). This is particularly important since seedling growth is maintained by seed mineral nutrient reserves until root uptakes commences supplying soil nutrients (Muhammad et al. 2015).

Seed priming with with micronutrients has of late gained momentum in various research programs (Kumar et al., 2020). Nutrient seed priming is widely used in parts of Asia with studies in Bangladesh, Nepal, India and Pakistan showing that NSP can increase maize yields by up to 70% after adding such nutrients as molybdenum, zinc, boron and phosphate to priming water (Harris et al., 2001). Harris et al. (2007) showed positive responses of chickpea and wheat to priming with ZnSO4 in moderately Zn-deficient soils. In addition to yield increases following priming with ZnSO4, grain Zn concentration increased by 12% in wheat and 29% in chickpea. Most importantly, NSP was very cost-effective, with net-benefit-to-cost ratios of 7.5 for wheat and 7.80 for chickpea observed. Similarly, Haider et al. (2020) observed improved grain yield and grain content following seed priming with 0.01M Zn and 0.05M Zn solution as contrast with control. In another study, Zulfigar et al. (2020) demonstrated that Fe seed priming was the most effective method in improving the grain yield and benefit-cost ratio in conventional and zero tillage systems in comparison to soil and foliar application. Likewise, Sime and Aune (2019) reported improved agronomic performances and economic returns in maize production after combining seed priming and fertilizer micro-dosing in Ethiopia.

The benefits of NSP are more pronounced during the early growth stages. For instance, Badiri et al. (2014) showed that total biomass was increased whilst time to emergence was reduced after priming with iron (Fe), zinc (Zn) and manganese (Mn) for broadleaf plantain. Rahman et al. (2014) reported high maize emergence in a study after sowing primed seeds compared to non-primed seeds in Bangladesh. Similarly, primed cotton and maize seed resulted in higher germination percentages at low water potentials than non-primed seeds in a study conducted in Zimbabwe (Murungu et al., 2005). El-Sawi et al. (2010) showed improved results on growth and yield of tomato. Similarly, Uche et al. (2016) indicated that seed priming improved germination of green-pepper seeds. Growth parameters such as germination mean germination time and coefficient of velocity of germination were improved after seed priming mountain rye (Ansari et al., 2013). Nutrient seed priming is thus a cheaper way of adding micronutrients compared to expensive methods like foliar spraying. The high grain concentration of essential elements such as Zn may also be a cheaper way of improving human nutrition (Dimka and Bindraban, 2016). Other benefits of seed priming include increased seedling tolerance to stress. For instance, studies by Iqbal et al. (2020) showed reduced negative effects of salinity through seed priming with CuSO4 and ZnSO4 at two concentration levels (100 & 200ppm).

The potential for acceptance or adoption of this technology is therefore high, given that on-farm seed priming is a widely accepted technology for improving yield in other resource poor farming areas of the world (Harris et al., 2001). In on-farm experiments in some resource-poor farming areas of Zimbabwe, farmers reported faster/earlier emergence, less re-sowing and more vigorous plants (Harris et al., 2001). Earlier, Witcombe and Harris (1997) reported widespread success of seed priming by farmers in India and Zimbabwe.

While there is a lot of scientific data asserting the effectiveness of NSP in some countries (Kumar et al., 2020), little effort has been made to refine and standardize this technology for the marginal soils of resource-poor farmer fields in South Africa despite the poor micronutrient status of their soils. This is particularly important since response of seed germination and early seedling growth to NSP may vary with crop, variety, priming agents used, concentration and priming duration (Stephen et al. 2018). Consequently, there is need for optimizing the concentrations and priming duration of these nutrients and hence establish a proper priming protocol in order to prevent possible nutrient toxicity that can hinder germination. This is particularly important since little information on the optimization of Zn, Mo and B seed priming is available in South Africa.

The study’s hypothesis is that NSP with optimum concentrations and appropriate priming duration of limiting micronutrients will improve maize seed germination, emergence and stand establishment. The objective was to determine the optimum concentrations and appropriate priming duration for various limiting micronutrients to improve maize stands and seedling vigour.

2. Materials and methods

Laboratory and glasshouse experiments were carried out at the University of Limpopo, Department of Soil Science Laboratories and Green Biotechnologies Research Centre of Excellence respectively, (23°53'10"S, 29°44'15"E) to determine the effects of micronutrient seed priming on maize (Zea mays) germination and seedling growth in some micronutrient deficient soils. Soils for the glasshouse study were collected from Ofcolaco, 43 km south-east of Tzaneen town (24° 6’0"S, 30° 23’0"E) in South Africa (Figure 1). The landscape is covered by a broadleaved deciduous forest and is characterised by clay loam soils of Hutton form according to South Africa classification system (Soil Classification Working Group, 1991) or Rhodic Ferralsol (IUSS Working Group WRB, 2006). The climate is classified as a humid subtropical (dry winter and hot summer). The area receives an annual average rainfall of 700 mm with minimum and maximum average temperatures of 9 °C and 30 °C, respectively.

2.1. Soil sampling and characterisation

Soils for the glasshouse study were randomly collected from a 1 ha plot. Maize had been grown on the sampling site successively for more than five years. Twenty soil samples collected from the top 0.15 m were mixed to make a composite sample. The soil samples were taken to the laboratory, air-dried at room temperature, subdivided into 3 sub-samples and sieved through a 2 mm sieve for subsequent analysis.

The soil was characterized for physical and chemical properties, such as soil pH, texture, exchangeable bases (Ca, Mg, K and Na), soil organic carbon (SOC), electrical conductivity (EC), cation exchangeable capacity (CEC), P, Zn, B, and Mo content. Soil pH was measured in water at a soil water ratio of 1:2.5 using a pH meter (model pH 25, Crison Instruments, Johannesburg) (Okalebo et al., 2000). EC was determined from the suspension of soil water ratio same as pH, measuring the electrical resistance using conductivity meter or probe. Particle size distribution was determined using the pipette method after oxidizing SOM with hydrogen peroxide (Gee and Or, 2002). Soil organic carbon was determined using Walkley-Black chromic acid wet oxidation method (Nelson and Sommerr, 1996). Exchangeable bases (Ca, Mg, Na, and K) were determined by treating the samples with ammonium acetate buffered at pH 7.0 while CEC was determined using 1M ammonium acetate (NH4OAC) saturation solution (Hendershot et al., 2008). Phosphorus was determined following Bray 1 method. The multiple soil extractant (Di-ammonium EDTA) was used for extraction of Zn, B and Mo concentration from the soil samples (Okalebo et al., 2000).

2.2. Laboratory experiment

The treatments used for the laboratory experiment included three different priming solutions (zinc sulphate (ZnSO4), sodium molybdate (Na2MoO4) and boraxic acid (H3BO3)) at five different concentrations i.e. 0% (control), 0.01%, 0.05%, 0.1% and 0.5% primed for three different durations (24 h, 12 h and 8h) replicated three times. Seeds for the control treatment were soaked in distilled water for the same duration. Sixty maize seeds (DKC80) were soaked in 200 ml solution in the dark for each specific
duration. The leachates (salts) from the seed coat were removed by rinsing
the seeds three times with deionized water. Afterwards, the seeds were
dried at room temperature for a minimum period of 1 h (Harris et al.,
2007). Thereafter, 60 seeds from each priming solution were laid between
layers of germination paper placed in petri dishes. The petri dishes con-
taining the primed seeds were then placed in an incubator set at 25 °C for
10 days. The germination paper was irrigated with 10 ml of distilled water
using a pipette every other day. Seed germination was recorded on a daily
basis. The seeds were considered to have germinated when at least 2 mm
long radicle protrudes through the seed coat. Days to germination was
recorded when 50% of the seeds had germinated.

2.3. Data collection

Data were recorded on final germination percentage (GP), germination
rate (GR), mean germination time (MGT), days to germination (DG) and
coefficient of velocity of germination (CVG). Equations in Table 1 were used
to determine the characteristics of seedlings for the laboratory experiment.

2.4. Glasshouse experiment

The results of the laboratory experiment were used to formulate the
treatments for the glasshouse experiment. This was done by selecting the
best three best performing micronutrients concentrations for each
nutrient and two durations. A completely randomized design (CRD) laid
out in a 9 × 2 factorial treatment structure with 3 replications and a
control (hydro-priming) was used for the glasshouse study. The treat-
ments consisted of three priming solutions (zinc sulphate (ZnSO4), so-
dium molybdate (Na2MoO4) and boric acid (H3BO3) at 9 nutrient
priming concentrations (Zn 0.01%, Zn 0.05%, Zn 0.1%, Bo 0.01%, Bo
0.05%, Bo 0.1%, Mo 0.01%, Mo 0.05%, Mo 0.1%) and a control (0%) primed at two different durations i.e. 12 and 24 h in three replicates.
Seed priming was done following the same procedures as those used for
the laboratory experiment.

Plastic pots with a diameter of 25 cm were filled with soil. Three seeds
were sown in each pot at depth of 0.03 m. Each plant was irrigated with
500 ml tap-water on a three-day interval. The experiment was run for a
period of 4 weeks (i.e. up to the V3 – V5 stage of maize) before termi-
nation. Average air temperatures of 25 ± 3 °C were maintained in the
glasshouse throughout the experiment.

The following data were collected: days to emergence (DE), chloro-
phyll content index (CCI), stem diameter (SD), seedling height (SH),
seedling weight (fresh (FSW) and dry (DSW)) and final root length (RL).
Stem diameter was measured with a digital Vernier calliper, chlorophyll
content with chlorophyll meter (MINOLTA SPAD-502) and seedling
height was measured using a measuring tape. On the final day of the

Table 1. Equations used to determine selected seedling characteristics.

| No | Equation | Reference |
|----|----------|-----------|
| 1  | GP = \( \frac{\text{Number of normal germinated seeds}}{\text{total number of seeds}} \) × 100 (1) | (Zahedifar and Zohrabi, 2016; ISTA, 1996) |
| 2  | GR = \( \sum_{i=1}^{J} \frac{n_i}{d_i} \) (2) | (Zahedifar and Zohrabi, 2016; ISTA, 1996) |
| 3  | MGT = \( \frac{\sum_{i=1}^{J} n_i d_i}{\sum_{i=1}^{J} n_i} \) (3) | (Zahedifar and Zohrabi, 2016; Ellis and Roberts, 1981) |
| 4  | CVG = \( \frac{G_1 + G_2 + G_3 + \ldots + G_n}{1 \times G_1 + 2 \times G_2 + \ldots + n \times G_n} \) (4) | (Zahedifar and Zohrabi, 2016; Scott et al., 1984) |

Where \( n \) is the number of seeds emerged on an \( i \)th day and \( d \) is the number of days counted from the beginning of the experiment. \( J \) is set to 7 days in this experiment, \( n \) is the number of seeds germinated on day and \( d \) is the number of days from the beginning of the experiment, \( G_1 - G_n \) is the number of germinated seeds from the first to
the last day. GP = Germination percentage, GR = Germination rate, MGT, = Mean germination time, CVG = Coefficient of velocity of germination.
experiment, seedlings were uprooted and washed off over a 53 μm sieve to remove all the soil from the roots. Root length was measured with a ruler whilst FW was measured with a weighing balance. Dry seedling weight mass after drying the seedlings at 65 °C in a forced air oven until constant weight was achieved.

2.5. Data analysis

Analysis of variance (ANOVA) was carried out to test for effect of treatments on maize development and yield parameters (Gomez and Gomez 1984) using JMP 14.0 statistical software (SAS Institute, Inc., Cary, NC, USA). Mean comparison was performed using the least significant difference test (LSD) at α = 0.05.

3. Results

3.1. Initial characterization

The soil texture was sandy clay loam, with a very low organic carbon content of <1% (Table 2). All the three micro-elements were below the threshold levels for maize production. Generally, soils containing less than 1.5–2 mg kg⁻¹ Zn, less than 1–2 mg kg⁻¹ Bo and less than 0.1–0.2 mg kg⁻¹ Mo are classified as potentially Zn, B and Mo deficient respectively (FSSA, 2007a).

3.2. Effect of boron seed priming on maize seed germination

Both priming duration and boron concentration had significant (P < 0.05) effects on GP, GR, CVG, days to germination (DG) and mean germination time (MGT). The interaction between these factors was also significant (P < 0.05). The lowest GP (87.33%) was observed after seed priming with 0.5% B for 24 h. However, no significant differences were observed in GP from 0 up to 0.1% B at both durations (Figure 2a). Within each B concentration level including the control, seeds primed for 24 h had the highest GR than both the 8 and 12 h durations except for the 0.5% B concentration where priming for 24 h resulted in the lowest GR (Figure 2b). Priming seeds with B at 0.5% for 24 h significantly slowed down the overall GR as compared to seeds primed with water (0%). Hence, seed primed with B at 0.01% for 24 h (47.53% per day) increased GR, but was not significantly different from seed primed with 0, 0.05 and 0.1% B for 24 h (Figure 2b). The seeds primed with B for 24 h at 0.01, 0.05 and 0.1% levels obtained a faster CVG of 0.85, 0.84, 0.84 and 0.8, respectively, while the lowest was achieved for seeds primed for 8 h at 0.05%, 12 h at 0.01% and 24 h and 0.5% (0.46, 0.44 and 0.38, respectively) (Figure 2c). The earliest germination was observed for seeds primed with B for 24 h at 0, 0.01, 0.05 and 0.1% (one day), these treatments were significantly different from the rest of the treatments. However, the rest of the treatments were not significantly different from one another and germinated after two days (Figure 2d). Mean germination time ranged between 1.17 and 2.66 days across all interactions. The longest MGT was for seeds primed with B at 0.5% for 24 h and the shortest was for seed primed for 24 h at 0, 0.01, 0.05 and 0.1% B (Figure 2e).

3.3. Effect of zinc seed priming on maize seed germination

Zinc seed priming duration and concentration levels had significant (P < 0.05) effects on GP, GR, CVG, DG and MGT. The interaction between seed priming duration and Zn concentration levels on GR, CVG, DG and MGT was statistically significant (P < 0.05) but not statistically significant for GP (Table 4). Seed priming with Zn at 0.01% for 24 h significantly increased the overall GR (48.06% per day) as compared to the control (21.62 % per day) (Figure 3a). A faster CVG of 0.87, 0.86 and 0.85 were obtained for seeds primed with Zn for 24 h at 0, 0.01, 0.05 and 0.1% respectively. The slowest was observed for seeds primed for 8 h at 0% (Figure 3b). The earliest germination resulted after seed priming with Zn for 24 h at all concentration levels (one day). Seeds primed with Zn for 12 h and 8 h (except at 0.05% Zn) germinating after an average of 2 or 3 days (Figure 3c). Mean germination time ranged between 1.17 and 3.21 days across all interactions. The longest MGT was observed for seeds primed with Zn for 8 h at 0% and the shortest was observed for seed primed for 24 h at 0.05% (Figure 3d).

3.4. Effect of molybdenum seed priming on seed germination

Seed priming duration and Mo concentration levels had statistically significant effects (P < 0.05) on GP, GR, CVG, DG and MGT. The interaction between seed priming duration and Mo concentration levels on CVG, MGT and DG was not statistically significant (P > 0.05) while the effect on GP and GR was statistically significant (P < 0.05). Seed priming for 24 h regardless of concentration levels resulted in higher GP compared to priming for 8 and 12 h. The lowest GP (80%) was observed for seeds primed with Mo at 0.5% for 12 (Figure 4a). Similar to observation for GP, seeds primed with Mo had a similar GR for the duration of 24 h regardless of priming concentration (Figure 4b). However, seed priming with Mo for 12 h at 0.5% and for 8 h at 0.5% resulted in the slowest GR of 18.62 and 24.36% per day respectively. Seed priming with 0.5% Mo for 24 h resulted in the lowest CVG whilst NSP with Mo at 0.01% for 24 h resulted in the fastest CVG (Figure 4c). Seeds primed with 0.01% Mo for 24 h resulted in the short germination time (1 day) and seed primed with 0.5% Mo for 12 h resulted in the longest germination time (2.47 days). All the seeds primed for 24 h with Mo germinated after 1 day (the earliest) at all concentration levels (Figure 4d). Meanwhile, seeds primed with 0.5% Mo for 12 h were the latest (2.67 days) to germinate and resulted in the lowest germination. Similarly, seeds primed for 24 h had the shortest MGT of 1 day (Figure 4e).

3.5. Micronutrient seed priming and priming duration effects on the performance of maize seedling under glasshouse conditions

Nutrient seed priming had significant effects (P < 0.05) on the number of days to emergence (DE), dry seedling weight (DSW), chlorophyll content index (CCI) and seedling height (SH) whilst its effects on seedling diameter (SD) and fresh seedling weight (FSW) were not significant (P > 0.05). Priming duration had significant effects on all parameters except CCI and root length (RL) (Table 3). There was significant interaction effects between nutrient seed priming and priming duration on DSW, SH and RL (P < 0.05).

Both nutrient seed priming and priming duration had significant effects on DE (P < 0.05), whilst interaction between the two factors was not

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**Table 2. Initial soil characterization.**

| Soil properties | Clay | Silt | Sand | Soil texture | Zn | B | Mo | EC | pH | Organic carbon | Total N | P | Ca | Mg | K | Na |
|-----------------|------|-----|------|------------|----|---|----|----|----|----------------|--------|---|---|----|---|---|---|
|                 | 26%  | 15% | 59%  | Sandy clay loam | 0.364 mg/kg | 0.362 mg/kg | 0.0072 mg/kg | 43.8 μS/m | 6.57 H₂O | 1.072% | 0.05% | 38.718 mg/kg | 764.25 mg/kg | 203.406 mg/kg | 140.176 mg/kg | 11.734 mg/kg |

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significant. Seedlings primed with Bo at a concentration of 0.01% emerged earliest, whilst the non-nutrient primed control resulted in the latest emergence (Table 4). However, the difference between the 0.01% and 0.05% concentration for all three nutrients was not significant, whilst the 0.1% concentration for all the three micronutrients was similar to the control. Priming for 12 h resulted in more DE than priming for 24 h regardless of priming concentration. Fresh seedling weight was only significantly affected by priming duration. The fresh weight of seedlings primed for 24 h was double that of seedlings primed for 12 h (Table 4). Nutrient seed priming significantly affected CCI, whilst priming duration had no effects. Whilst priming with Bo 0.01%, Mo 0.1% and Mo 0.01% resulted in the highest CCI, this was however similar to the control (Table 4). However, seeds primed with Bo 0.01% and Zn 0.1% had the lowest CCI.

Only priming duration significantly affected (P < 0.05) fresh seedling weight (Table 5). Priming for 24 h resulted in more than double the fresh weight observed after priming for 12 h. In contrast, the interaction between nutrient priming and priming duration significantly affected DSW (Table 4). Priming for 24 h resulted in significantly higher seedling dry weight than priming at 24 h except for the control where there was no difference in seedling dry weight due to the two priming durations (Figure 5a). Seeds primed with Bo at 0.1% and 0.05 for 12 h resulted in

Figure 2. Effect of priming duration and boron concentration levels on (a) germination %, (b) germination rate, (c) coefficient velocity of germination, (d) days to germination and (e) germination time.
the lowest DSW. The seedling dry weights for these two treatments were lower than those for the control. The rest of the treatments resulted in similar seedling dry weights to that observed for the control. Whilst seedlings primed for 24 h resulted in significantly higher seedling dry weight than those primed for 12 h, there were very few differences among the priming treatments. However seeds primed with Bo at 0.01% for 24 h resulted in the highest seedling dry weight (see Figures 6 and 7).

Seed priming for 24 h resulted in taller plants than those from seeds that were primed for 12 h except for the control were the opposite was true (Figure 5b). For the 12 h priming duration, most NSP treatments performed similarly to the control except for Bo 0.05% and Bo 0.1%, which were both outperformed by the control. In contrast, for the 24 h priming duration, no differences were observed among the nutrient priming treatments. Moreover, all nutrient priming treatments outperformed the control.

There were generally little differences in root length between seed primed for 12 h and those primed for 24 h except Zn 0.05%, Zn 0.2% and Bo 0.1% where priming for 24 h resulted in significantly higher root length (Figure 5b). Seeds primed in water only for 24 h resulted in the shortest roots.

4. Discussion

4.1. The effect of micronutrient seeds priming on selected seed germination parameters

Fast seed germination and emergence are important for maize growth because they improve its competitive ability against weeds for water and nutrients. Seed priming with micronutrients (Zn, B and Mo) improved mean germination time (MGT), germination percentage (GP), the Coefficient of velocity of germination (CVG), germination rate (GR) and reduced days to germination (DG). Similarly, Rahman et al. (2014) also indicated that higher GP, decreased MGT and increased CVG were observed for seeds primed with micronutrients This could be due to the synthesis of DNA, RNA and proteins during NSP (Afzal et al., 2008). In a more recent study, (Ullah et al., 2019), observed improved mean germination/emergence time and seedling growth of chickpea with Zn seed priming in 0.001 and 0.0001 M Zn solution except 0.01 M Zn which caused toxicity. This was attributed to the involvement of Zn in radicle development and in the early stages of coleoptile growth and in auxin synthesis (Ozturket al. 2006; Ullah et al., 2019). The improvement in germination parameters of NSP treatments as compared to the control (only water primed) could also be due to the increased nutrient content in the seeds. During priming, proteins like the beta subunit of the globulin is increased, lipid peroxidation is reduced and antioxidative activities are enhanced as they are responsible for germination in the seeds (Varier et al., 2010). After drying the primed seed upon re-absorption of water rapid growth is observed and radicle and plumule appear earlier. Moreover, Afzal et al. (2008) observed higher levels of amylase activity, ascorbate and phenolic contents in nutrient primed seeds whilst Chen and Arora (2011) conclude that seed priming endo-β-mannanase activity, which weakens the endosperm and hence promotes germination. Moreover, NSP increases the germination by increasing the activity of enzymatic antioxidants, superoxide dismutase (SOD) and peroxidase (POD) which helps in utilization of protein and carbohydrate during germination process Kiran et al., (2012); Shrestha et al. (2019).

One of the important determinants of the effectiveness of NSP is priming duration. In this study there was significant interaction between priming concentration and priming duration for most of the parameters measured. This finding was supported by Dezfuli et al. (2008), Yohannes and Abraha (2013) and Stephen et al. (2018) who reported that seeds primed for a longer period at lower concentration levels performed better than other treatments. Similarly, Johnson et al. (2005) and Guan et al. (2009) also observed that at higher

![Figure 3. Effect of priming duration and zinc concentration levels on (a) germination rate, (b) coefficient velocity of germination, (c) days to germination and (d) germination time.](image-url)
concentration levels, maize germination decreased while at lower concentration it was elevated. Similar observations were made by Ullah et al. (2019) who observed lower germination and reduced growth and development of chickpea at higher application of Zn due to decrease in gibberellic acid and zeatin in germinating seeds. The decrease in germination and germination rate for seeds primed for a longer period at higher concentration levels could also be due to increases in nutrient toxicity in the seed coat. Toxicity alters the enzymes of the nucleus and metabolism causes protein metabolism to interfere with hormonal balance and reduces the utilization of seed food reserves during germination and germination time.

Table 3. ANOVA for the effect of nutrient seed priming, priming duration and their interaction on days to emergence, seedling weight, final root length, seedling height, shoot diameter and chlorophyll for maize in glasshouse.

| Source                  | DF | DE days | FSW grams | DSW grams | CCI   | SD cm | SH cm | RL cm |
|-------------------------|----|---------|-----------|-----------|-------|-------|-------|-------|
| Nutrient priming        | 9  | <.0001* | 0.1652    | 0.0456*   | 0.0125*| 0.4537| 0.0123*| 0.5350 |
| Priming duration        | 1  | <.0001* | <.0001*   | <.0001*   | 0.5462| 0.0059*| <.0001*| 0.1000 |
| Priming × Duration      | 9  | 0.4347  | 0.3718    | 0.0196*   | 0.1313| 0.5774| 0.0046*| 0.0207* |

Significantly different (*), days to emergence (DE), fresh seedling wet weight (FSW), dry seedling weight (DSW), chlorophyll content (CCI), seedling diameter (SD), seedling height (SH), root length (RL).
germination (Yohannes and Abraha, 2013). Priming duration is therefore a critical factor to consider in NSP technique.

4.2. Effect of micronutrient seed priming of seedling growth

Seedling emergence and establishment are the key processes in the survival and growth of plants. Seedling establishment was improved for seeds primed with micro-nutrients as compared to seeds primed with water. Seeds primed with micro-nutrient solutions resulted in higher, longer, thicker and heavier seedlings. Liz/C19 P. Lizarraga-Paulín et al. (2013) and Zeng et al. (2012), support these findings with similar conclusions that NSP improvement seedling height, length, thickness and weight on maize and soybean respectively. The accessibility of micronutrients in the seeds are vital for protein synthesis and enzymes responsible for seedlings to effectively utilize the other nutrients in the soil. This consequently improved seed germination and seedling establishment. Moreover, micronutrients such as Mo and B are required for effective use of NPK nutrients by variety of crops (Kaiser et al., 2005; Singh et al., 2014). In addition, these improvements in growth and developments for seedlings primed with solutions could be due to the earlier uptake of solutions which activated the germination process. Seeds which, are soaked in water for a particular duration and dried before seminal root protrusion can develop and grow faster (Sozharajan and Natarajan 2014). In this study, seeds primed with micronutrients emerged earlier than the control, which could have increased their establishment resulting in superior use of nutrients as evidenced by better seedling weight, shoot length and root length. Moreover, the increase in the root length of seeds primed with micronutrients could be due to activation of cell respiration and cycling during priming. Activation of cell respiration and cycling, repair of macromolecules, assimilated materials translocation and weakening of seed coat structure results in faster root emergence (Vasquez-Ramos and Sanchez, 2004; Cantliffe et al., 1984). Nutrient seed priming especially at low concentration increased the content of chlorophyll compared to the higher concentration and the control. This could have enhanced the photosynthesis of the seedling and hence better growth. In this study the best treatment was B 0.01%. This treatment resulted in the earliest seedling emergence, highest seedling weight, root length and seedling

### Table 4. The effect of concentration levels, duration and interaction of concentrations levels and duration period on days to emergence, seedling weight, final root length, seedling height, shoot diameter and chlorophyll content index for maize in glasshouse.

| Treatments | DE days | FSW grams | DSW grams | CCI | SD cm | SH cm | RL cm |
|------------|---------|-----------|-----------|-----|-------|-------|-------|
| Control    | 9.3a    | 13.47bc   | 1.28c     | 12.02bde | 0.44b  | 18.96c | 33.05bc |
| Zn 0.01%   | 4.49f   | 17.00b    | 2.10ab    | 12.88bc | 0.47ab | 28.54bc | 45.30ab |
| Zn 0.05%   | 5.58bc  | 14.45bc   | 1.86bc    | 7.48bcd | 0.45bc | 28.19bc | 4.049ab |
| Zn 0.1%    | 8.67a   | 15.72bde  | 2.07bc    | 7.00bd  | 0.47b  | 28.62bc | 39.34ab |
| Be 0.01%   | 4.73bc  | 19.11ab   | 2.44a     | 15.41a  | 0.42b  | 32.67ab | 52.28ab |
| Be 0.05%   | 6.17b   | 13.97bc   | 1.95ab    | 13.95ab | 0.37b  | 26.57bc | 36.93ab |
| Be 0.1%    | 8.83*   | 9.63*     | 1.54ab    | 5.32d   | 0.5ab  | 23.35cd | 37.07ab |
| Mo 0.01%   | 5.33bc  | 20a       | 2.37a     | 14.93a  | 0.55a  | 33.88a  | 47.17ab |
| Mo 0.05%   | 5.83bc  | 13.97bc   | 2.03ab    | 13.25ab | 0.45bc | 27.08bc | 37.62ab |
| Mo 0.1%    | 8.5a    | 15.2abc   | 2.02ab    | 15.18a  | 0.45bc | 31.16ab | 46.15ab |

Significantly different (*), days to emergence (DE), fresh seedling wet weight (FSW), dry seedling weight (DSW), chlorophyll content index (CCI), seedling diameter (SD), seedling height (SH), root length (RL).

Figure 5. Effect of nutrient priming and priming duration on dry seedling weight (Error bars indicate standard error).
height. Coincidentally, this treatment also resulted in the highest chlorophyll content (Table 4). Similarly, Rehman et al. (2013) observed higher leaf chlorophyll content from priming solution had very low levels of boron (0.001%).

5. Conclusion

Nutrient seed priming with Zn, Mo and B improved the germination and early seedling growth of maize compared to priming with water only. However, priming duration is an important consideration to make. Priming at lower nutrient concentrations but for longer duration gave the best results compared to priming at higher concentrations for the shortest duration. Nutrient seed priming with B at 0.01% for 24 h was the most effective treatment in improving seedling growth under glasshouse conditions. It is however necessary to carry out further experiments on the effects of associations of these micronutrients since they may interact with each other in their effects on maize growth.

Declarations

Author contribution statement

A. D. Nciizah: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

M. C. Rapetsoa: Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

I. I. C. Wakindiki: Conceived and designed the experiments; Analyzed and interpreted the data; Wrote the paper.

M. G. Zerizghy: Analyzed and interpreted the data; Wrote the paper.

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