



Research Article

Volume 26 Issue 5 - March 2022

DOI: 10.19080/ARTOAJ.2022.26.556349

Agri Res & Tech: Open Access J

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GA3 and KNO₃ Efficiency of Breaking Dormancy In Wild Brassicaceae (*Brassica napus* and *Raphanus raphanistrum*) Seeds for Enhanced Phytoremediation



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Submission: May 16, 2022; Published: June 10, 2022

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Abstract

Brassicaceae family are largely stress resistant to extreme environmental conditions particularly heavy metals laden soils. They are found in post-harvest agricultural field and sometimes during farming season costing economic loss to farmers. The species, *Raphanus raphanistrum* (wild radish) and *Brassica napus* (canola) were collected from agricultural fields within the University of Eldoret farmlands. The morphometric characteristics, longitudinal length, transverse length (width), perimeter, areas, and seed weight were assessed. Matured seeds within siliques were sundried in a glasshouse (25-45°C) for several days until constant weights were obtained. About a hundred seeds of each, wild radish and canola were mechanically removed and portioned into five germination test groups. The seeds presoaked in distilled twenty-four hours before treatment with plain water (control), concentrated acid solutions (H₂SO₄ and HCL 5 minutes and 30 minutes, respectively), Chilling at 4°C, 0.25mg/mL Gibberellic Acid (GA3), and 0.1% alkaline (KNO₃). Pretreated seeds were transferred into sterilized petri dishes containing double-layer filter papers. The germination experiments were carried out in biotechnology lab at 20-25°C under normal lightening. In all setups, germination was effective in *Brassica napus* yielding 80% and 90% germination rate for GA3 and KNO₃, compared to *Baphanus raphanistrum*, 70% and 10%, respectively. Priming with H₂O, H₂SO₄, HCL, and chilling were ineffective in this trial.

Keywords: Induced Germination; Breaking Dormancy; Seed Priming

Introduction

Cruciferae are economically essential crop family that includes fodder crop, oilseed plants, and vegetables for food, organic fertilizers, and biofuels. Many species of Brassicaceae are tolerant to environmental stress conditions and agrochemicals application such that they are widely used as model species [1]. There are over 3000 species from more than 300 genera of the mustard family, amongst them is the most used experimental plant, *Arabidopsis thaliana*. This simple angiosperm has paved ways to understanding growth and development of plants [2 Table 1: Morphological characteristics of *raphanus raphanistrum*]. Numerous studies have been conducted to traceably establish the origin of the mustard family with many indicating Eurasia as the family origin before it was spread to the northern hemisphere and other parts of the world. The ancestors of brassicaceae are evidently believed to have originated from northeastern Mediterranean and later spread to Asia and Europe evidenced by the biogeographic events

in the area [3]. *Brassica napus* is deliberated to have formed around 5000-10, 0000 mya most likely originating from the interspecific hybridization of the genotypes *Brassica rapa* and *brassica oleracea* [4]. It is one of the most cultivated medicinal crops in Eurasia and Saharan Africa, and also has attracted huge commercial value from its enriched oilseed potential to produce cooking and renewable energy [5].

Raphanus raphanistrum (wild radish), a widely known distributed weeds and it is understood to belong to the brassica plants though with some disputes of any link [6]. It has been reported as a troublesome weed for cereals in some parts of Australia, growing sporadically from multitude of seeds produced seasonally and interferes with yields of production [7,8]. It has herbicidal resistance to some agrochemicals use around the world, including Acetolactate, chlorosulfuron, and metsulfuron-methyl [9-11]. Wild radish observed some dormancy in the soil seedbank

by physical restriction such as climatic conditions. The siliques provide additional adaptive mechanisms maximizing endurance during dormancy [12]. Wild radish also contains healthy and nutritious and bioactive compounds comprising of phenolic and of wild radish in different areas around the world to treat different health conditions [15,16].

hydroethanolic extracts that have supplementary antioxidant potential that can be added to human diet [13,14]. The bioactive compounds in *Raphanus raphanistrum* have long been used for the medicinal values. Several studies have reported different uses

Materials and Method

Seeds collections

Table 1: Morphological characteristics of *raphanus raphanistrum*.

Species	Morphological Characteristics				
Radish (RR)	Weight (gram)	Length (mm*)	Width (mm)	Perimeter (Sq.mm)	Area (Sq.mm)
WRR1	0.06 ± 0.00	1.51 ± 0.00	1.28 ± 0.02	4.36 ± 0.04	1614.51 ± 32.16
WRR2	0.06 ± 0.01	1.50 ± 0.01	1.17 ± 0.03	4.28 ± 0.00	1512.45 ± 33.19
WRR3	0.05 ± 0.02	1.50 ± 0.03	1.17 ± 0.02	4.22 ± 0.02	1472.29 ± 34.18
WRR4	0.05 ± 0.00	1.57 ± 0.01	1.16 ± 0.01	4.31 ± 0.07	1523.47 ± 31.16
WRR5	0.05 ± 0.01	1.48 ± 0.02	1.13 ± 0.01	4.11 ± 0.05	1386.36 ± 35.17
Mean	0.06 ± 0.02	1.51 ± 0.01	1.18 ± 0.01	4.25 ± 0.04	1501.81 ± 33.11

Table 2: morphological characteristics of *brassica napus*.

Species	Morphological Characteristics				
Canola (BN)	Weight (gram)	Length (mm)	Width (mm)	Perimeter (sq.mm)	Area (sq. mm)
CBN1	0.03 ± 0.01	0.88 ± 0.02	0.72 ± 0.04	2.56 ± 0.05	559.72 ± 32.78
CBN2	0.03 ± 0.02	0.99 ± 0.01	0.93 ± 0.03	2.94 ± 0.07	729.95 ± 37.76
CBN3	0.03 ± 0.00	0.98 ± 0.02	0.90 ± 0.05	2.99 ± 0.08	768.39 ± 37.77
CBN4	0.03 ± 0.01	0.93 ± 0.01	0.94 ± 0.02	2.94 ± 0.07	741.62 ± 36.76
CBN5	0.03 ± 0.00	0.91 ± 0.02	0.90 ± 0.06	2.88 ± 0.08	708.71 ± 38.77
Mean	0.03 ± 0.02	0.94 ± 0.02	0.88 ± 0.04	2.88 ± 0.07	701.68 ± 36.77

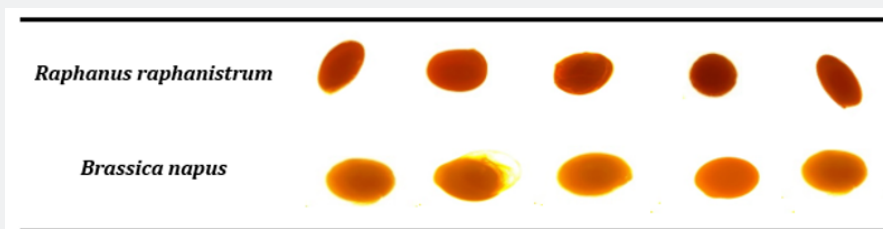


Figure 1: Images of Brassicaceae seeds showing their morphology.

Matured Seeds of *Raphanus raphanistrum* (wild radish) and *brassica napus* (canola) were collected from three wheat farms at the University of Eldoret during postharvest seasons where they grow wildly as weeds. The seeds were garnered while still green though fully matured/raped within the siliques. They were taken to the biotechnology laboratory, where they were stratified, labelled and spread on tray for sun drying. The seeds were weighed and transferred into the glasshouse for drying at temperature ranging 25-45°C. Some seeds were randomly selected to measure morphometrics (Figure 1) through a microscope including longitudinal length (length), transverse length (width), perimeter and area in millimeter. The seeds, together with their pods were measured every 24hrs for fourteen (14) days until a consistent dry weight was achieved, then they were mechanically removed

from the siliques and kept in paper bags at 20-25°C before experiment. Individual seed of each species was measured in five replications (Table 1 & 2) and were subdivided into experimental groups, comprising 100 seeds/species and treated with different priming techniques, chemical germination agents (CGA) in order to induce laboratory germination. The laboratory test materials and reagents included 100 by 15 mm petri dishes, doubled-layer tissue papers, wash bottles, 70% ethanol, 0.25 mg/L of gibberellic acid (GA3), 0.1% potassium nitrate (KNO3), concentrated hydrochloric (HCl) and sulfuric (H2SO4) acids. The priming techniques efficiency to break dormancy in the selected cruciferae seeds were measured using equations 1, 2 and 3, to calculate Germination Index, Time for 50% germination of seedlings, Mean Germination Time, respectively [17].

Germination index (GI) was used to compute daily germination counts for the 10day trial, adopting the Association of Official Seed Analyst method as indicated in equation1 [18].

The time required for 50% of germination of the seedlings (T50) to occur was calculated using equation 2 below (Coolbear, FRANCIS, & Grierson, 1984). Though, percent of the seedling germinated at half of the experimental time, that is, in 5 days was calculated.

Where N is the final number of germinated seeds, ni and nj are ti and tj, respectively, when ni <N/2 <nj.

Similarly, the mean germination (MGT) was computed according to equation3, where n is the number of germinated

seeds or emerging seedlings on day D, where D is the sum of days counted from the start of germination (Ellis & Roberts, 1981):

As indicated in Table 3 below, twenty (20) seeds of each crop were placed in 50 mL beakers and presoaked in distilled water H2O for 24hrs before treatment with chemical germination agents. The seeds were removed from the water and desiccated for 2-3 hours (hrs.) prior to treatment. The seeds were then transferred to petri dishes disinfected with 70% ethanol and filled with doubled-layer soft tissues. The seeds were unselectively divided into five (5) groups, that is, 20 seeds per petri dish (5 replicates); and treated with 10 mL of GA3, KNO3, HCl, H2SO4 and H2O in separate setups. Hydropriming using distilled H2O was the control treatments for all induced germination in these trials.

Table 3: result of priming of brassica napus and *Raphanus raphanistrum* using various induced germination agents.

Treatment	Time	Day	Brassica Napus					Raphanus Raphanistrum				
			Tg	GP	T50	GI	MGT	Tg	GP	T50	GI	MGT
H ₂ O	24hrs	10	0	0	0	0	0	0	0	0	0	0
HCl:H ₂ SO ₄	30:5 min	10	0	0	0	0	0	0	0	0	0	0
Chilling	72Hrs	10	6	30	20	6.55	1.67	9	45	25	10.94	1.11
GA3	24hrs	10	16	80	50	19.43	0.63	14	70	60	23.02	0.71
KNO3	24hrs	10	18	90	55	19.26	0.56	2	10	10	2.77	5

Tg=total seeds germinated, Day=numbers of days of trial, GP=germination percent, T50= percent germination at half of trial day, GI=germination index, MGT=mean germination time

Results

The results, Tables 1 & 2 showed simillar morphological characteristics within species of *Raphanus raphanistrum* and *Brassica napus*, respectively.

When compared one-to-one, the morphometrics of the species showed wider difference in their perimeters, lengths and weights, with *R. raphanistrum* showing dominance in all physical characteristics measured as seen in Figure 2.

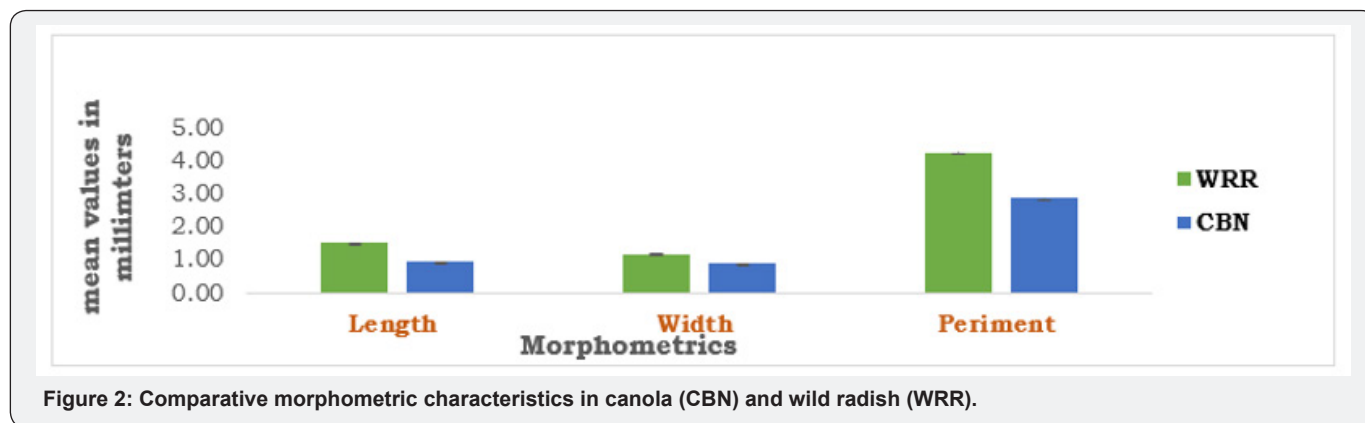


Figure 2: Comparative morphometric characteristics in canola (CBN) and wild radish (WRR).

From the experiment, treatments of brassica napus seeds with chemical germination agent performance rate were 90, 80, 30, 0 and 0 for KNO3, GA3, chilling, HCl: H2SO4, and H2O, respectively (Table 3). Similarly, treatment of *Raphanus raphanistrum* with same agents gave the following results: 70, 45, 10, 0 and 0 for GA3, chilling, KNO3, HCl:H2SO4, and H2O, respectively.

Discussion

Various seed priming techniques were used to induce germination in the two cruciferae species-brassica napus and *Raphanus raphanistrum*. Priming is a simple, less expensive and

maneuverable techniques that helps to improve seed germination, enhance early seedling emergence and stand formation against environmental stress conditions [19]. There are several means of seed priming, including osmo-priming, hydro-priming, chemical priming, physical priming, nano-priming and hormonal priming [20-22].

Chemical Priming

There are several chemical germination agents use to prime seeds including KNO3, H2O, HCl, H2SO4 using different concentrations for different seeds depending on the dormancy

and stress resistance. Halopriming using 0.1mM of KNO₃ used for the two species was very effective for brassica napus compared to *Raphanus raphanistrum* which agreed similar studies [23,24]. Also, use H₂SO₄ and HCl on the same crops. The seeds were exposed to the acids for a shorter time compared to KNO₃, that is, 5 minutes and 30 minutes, respectively. Hydrochloric and sulfuric acids blocked germinations in both species compared to the halochemical, KNO₃, and this is also true for other related studies [25,26]. On the contrary, other studies using same acids showed high germination rates compared [27-29,25].

Hormonal priming using gibberellic acid (GA3)

Gibberellic acid (GA3) is plant hormone that stimulates diverse metabolic synthesis in plant including seed germination, extensive growth, flowering; and its targeted site of bioactivity is the stamens [30,31]. A single concentration, 0.25mg/L, of GA3 was used to prime and induce germination in brassica napus and *Raphanus raphanistrum*. The results, 80 and 70, for CBN and WRR, respectively were the most effective priming agent overall, confirming similar finding/studies on GA3 use to enhance germination in Brassicaceae [32-34]. Though in certain plant species, and particular concentrations, GA3 slows germination of seeds [35-37].

Conclusion

The hormonal priming carried out by means of 0.25mg/L Gibberellic acid (GA3) was the most effective agent that induced germination in brassica napus and *Raphanus raphanistrum* followed chemical priming, 0.1M KNO₃ then chilling. Hydro-priming was completely ineffective to break the dormancy in the seeds. This finding is true for many seeds that go through secondary dormancy as results of building environmental resistant and adaptation.

Conflict of Interest

The authors declare that there are no known financial or personal competing interests that could have seemed to influence the work in this paper.

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DOI:10.19080/ARTOAJ.2022.26.556349

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