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Seed germination in maize (*Zea mays* L.) under the influence of different drinks flavors as a source of plant nutrients

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Key words: Maize (*Zea mays* L.), Fruit juices, Seed germination, Reducing sugars, Antioxidants

Abstract

In present work, effect of different nutrients in the form of various drinks flavors available in local market on plants was assessed on especially seed germination of maize (*Zea mays* L.) var., MMRI-Yellow and its early growth. Seeds germinated on different nutrient conditions like as T_0 (dH_2O), T_1 (MAY-sparkling fruit juice), T_2 (¼T_1), T_3 (Murree's Wheat Beer), T_4 (¼T_3), T_5 (Apple Mixed Juice), and T_6 (¼T_5) and maintained for 2-weeks. Among the morphological attributes maximum seed germination, seedlings biomass, lengths of plumules and radicals observed on T_0 and T_6 cultures and each are significantly lesser among seeds growing under alcoholic bears supplements (T_3 and T_4). The biochemical contents including chlorophyll (ab), total sugars and protein contents also showed same trend among the cultures ($p<0.05$). Meanwhile, chlorophyll b, total carotenoids, reducing sugars, antioxidants activity and phenolics observed maximum in plumules and radicals of seedlings grew in T_1 and T_3 cultures ($p<0.05$). These biocontents reduced among the dilutions to T_0 and T_6 cultures. These both cultures showed good seedling growth responses, whereas T_6 remained most growth supportive. It means that seed mobilization and its growth suppressed with supplementation of MAY-sparkling fruit juice (T_1) and Murree's Wheat Beer (T_3) even concentrated apple juice also (T_5). The T_1 and T_3 cultures showed impaired growth retardation during seed germination. Each of mixed fruit juice, non-alcoholic wine and alcoholic bears have performed significant role in seed germination and its initial subsequent growth.

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Introduction

The maize (*Zea mays* L.) crop has got 2^{nd} position among cereals after wheat in Pakistan (FAO, 2015). It is being used as human nutrition, livestock feed and its raw material in agro-based industries (Gwirtz and Garcia-Casal, 2014; Notenbaert *et al.*, 2013). A number of pressures exerted in the form of land limitation due to increasing population and plant pathogens are decreasing both of its quality and quantity. Consequently, approaches of conventional and modern biotechnology are emphasized. Conventional methods are economic and useful to get high yield from crops. On the basis of general characteristics of seeds that large sized seeds showed higher germination rate with its rapid emergence and further growth due to its large storage reservoirs in cotyledons (Alenca *et al.*, 2012; De Souza *et al.*, 1999; Seiwa and Kikuzawa, 1991).

The increasing rates of external applied environmental stresses are ultimately causing to decrease in crop yields including number of seeds or grain and its seed size. Large sized seeds have optimum raw material for its germination and initial growth of emerging shoots due to availability of substrate, enzymes and energy (ATP) for protein synthesis (Gallardo, 2002; Souza and Fagundes, 2014). *In-vitro* tissue cultures like as callus formation also dependent on seed size as well as its embryo's maturity (Akinyosoye *et al.*, 2015; Shah *et al.*, 2003). Meanwhile, a farmer is primarily believes that crop yields can be increased with applications of fertilizers, pesticides and good irrigation. For crop production, pesticide play an essential part to kill surface growing pathogen that makes possible to produce good yield to feed human as well as animals. Various varieties of different crops responses differently, when grown on the land with variant environmental biotic and abiotic stresses. The chemical stresses among abiotic stresses are known to exert negative impact on cell's metabolic processes, which results into reduction in vegetative growth of plant and its yield (Suzuki *et al.*, 2014; Verma *et al.*, 2016; Yumurtaci, 2015).

Meanwhile, little information about the effect of maize seed size on *in vitro* seed germination is available. Large sized seeds germinate nicely but small sized seed shows low germination rate. It could be due to low number of essential components of seeds. The deficiency of essential components may be cause of slow down or reduction of seed germination. Supplements of nutrients could be helpful to enhance the rate of seed germination.

The plants are able to retrieve their nutrients from soil medium or other sediments including *in-vitro* cultures. The surplus supply of inorganic nutrients in the form of hydroponics could be useful for plant growth while being costly. Almost similar typed nutrients including inorganic and organic molecules are present in the fruit juices. These juices are being the cheapest source of various nutrients in balanced form and are available in the commercial super markets.

Instead to use the fresh available commercial juices, their wastes can be used for this purpose after proper sterilization. Various fruit industries have a considerable waste of their final products. Mostly wastes are not recycled just thrown away on earth or even drainage out in the water. Both are toxics for animals and plants on the soil as well as in the water. It is being a source of environmental pollution.

The proper management of such wastes in the form of bio-nutrients could be useful source of nutrition for other organisms. Like as different fruit juices have attractive composition of various salts, vitamins and sugars. Each component of the juice is also variable in types and concentrations from juice to juice of fruits even including wines and bears also.

For a specific crop or even its variety, the selection of a fruit juice is a major task. For this purpose, the aim of this study is to measure the effects of local drinks flavor (mixed fruit juice, non-alcoholic wine and alcoholic bear) on subject of interest of seed germination, early growth rate of newly emergence and further their metabolic rates. A maize var., MMRI-Yellow is subjected for detailed evaluation of its seed germination on the basis of their biochemical changes under the influence of variant concentrations of local commercial fruit drinks.

Materials and methods

Plant seed materials

Present experiment was conducted in glass petri-dishes. The bases of dishes were lined with sterile whatman-40 filter paper. Seeds of maize (*Zea mays* L.) var., MMRI-Yellow collected from local grain market. The seeds were rinsed in 90% ethanol for 1 minute than stirred in 0.1% mercuric chloride ($HgCl_2$). After 10 minutes, seeds were washed with sterilized distilled water for 5-8 times. The 5 seeds were arranged on sterile filter paper and subjected to fruit juice treatments.

Application of fruit juices treatments

Three local drinks namely MAY-sparkling fruit juice (non-alcoholic), Murree's Wheat Beers (5.3% alcohol) and Multivitamins mix-fruit juice were purchased from local supermarket.

Different concentrations of each drink were raised by mixing with sterile distilled water as shown in table 1. Exact 3ml of each treatment was poured over filter-paper. The cultures were incubated at 25°C±2, 25% humidity and 18/6 h day and light photoperiod (light intensity ~2000 lux) for 2-weeks.

Table 1. Various treatments and its composition used for seed germination in maize (*Zea maize* L.).

#s.	Treatments	Compositions (Nutrient contents)
a.	T_0	Distilled water (dH_2O)
b.	T_1	*May Sparkling Fruit Juice (240 ml)*: Sodium 10 mg, carbohydrate 26 mg, sugar 26 mg, vitamin C 60%, iron 8%, calcium 2%.
c.	T_2	¼ T_1
d.	T_3	*Murree's wheat beer (240 ml)*: Calories 160, alcohol 5.3 %, fat 1 g, sodium 320 mg, carbohydrate 31 mg, dietary fiber 2 g, sugar 9 g, protein 4 g.
e.	T_4	¼ T_3
f.	T_5	Mixed juice (240 ml): 45 calories, fat 0.1 g, sodium 4 mg, potassium 101 mg, carbohydrate 11.6 g, dietary fiber 0.1 g, sugar 8.5 g, protein 0.1 g, vitamin A 1 %, vitamin C 106%, calcium 1 %, iron 2 %, thiamin 1 %, riboflavin 1 %, vitamin B_6 2 %, niacin 1 %, magnesium 1 %, phosphorus 1 %, copper 2 %, pantothenic acid 1 %.
g.	T_6	¼ T_5

Maize seed germination

Seeds of each culture were kept under moisture conditions with their respective concentration of drinks. After 2-weeks of seed incubation in plant growth room, seeds were examined day by day and number of germinated seeds were recorded. On 15th day, seedlings from each culture were harvested and subjected for fresh biomass measurements after counting germinated seeds. Both plumules and radicals lengths were taken and seedlings were dried in the electric oven at 72°C. On 3rd day, seedling's dry biomass was also measured.

Biochemical analysis of seedlings

Seedlings of each culture were subjected for analysis of various biochemical contents. Fresh seedlings were chapped into smallest fine pieces and agitated in 80% acetone. Mixture was incubated in dark at room temperature for overnight. Absorbance were taken at at 663 nm, 645nm, 453nm and 470nm and various chlorophyll contents and total carotenoids were calculated by following Arnon (chlorophyll) and Sumata *et al* (carotenoids) suggested formulas (Arnon, 1949; Sumanta *et al.,* 2014). For the estimation of total proteins, exact 1 ml extract of fresh seedlings was mixed with 2.5ml alkaline copper reagent and after 0.25ml follin-ciocalteau reagent also poured slowly than Absorbance was measured at 750nm (Dawson and Heatlie, 1984). Total sugars were analyzed by following Lee and Montgomery's method (1961). The 0.5ml sample mixed with 2.50ml conc. H_2SO_4 than 50µl 80% phenol was added. After 15 minutes OD was read at 485nm. Also for reducing sugars, 1ml of sample mixed with 1ml 2, 6-dinitrosalicylic acid (DNS) and heated for 5 minutes in boiling water bath. When cool-down, the absorbance was read at 540nm (Miller, 1959).

Estimation of enzyme activities

Among the seedlings cultures, antioxidants activity was determined by mixing 10µl of sample with reaction mixtures and it was capped with aluminum foil. It was heated on boiling water bath for 90 minutes.

After cooling, the absorbance was read at 734nm (Pisoschi and Negulescu, 2012; Prior *et al.*, 2005; Proestos *et al.*, 2013). *Protease* activities was estimate in 2.0ml sample after mixing with 1.5ml sodium phosphate buffer (pH 7.6) by following methods reported by Alef and Nannipieri, (1995) and Vágnerová and Macura, (1974).

Decontaminated conditions

All steps of this experiment are performed under sterilized conditions on laminar air-flow cabinet. The glass-ware i.e. petri-dishes, volumetric flasks, filter-paper and other stuff used in this experiment cleaned with vim and then sterilized at 121°C, 15 lbs/cm² in electric-autoclave for 15 minutes. Autoclaved stuff was dried in electric oven (65°C, for 4-6 hours) and was opened to use in laminar air flow cabinet.

Statistical analysis of data

Data was collected from each seedling cultures and subjected for treatment significance with analysis of variance (ANOVA) on CoStat (3.03) *CoHort* software, Berkeley (USA). Mean differences between treatments also calculated with Duncan Multiple Range (DMR) test at 5% (Behrens, 1997; Henley, 1983).

Results and discussion

The maize (*Zea mays* L.) cereal is cropped widely due to its increasing demand to reach human consumption. With the passage of time its quality and quantity is decreasing with changing environmental stresses especially seed health (Kansiime and Mastenbroek, 2016; Liu *et al.*, 2016). The seeds with poor health cause inhibition for its germination and remain susceptible to the attacks of pathogens and insects (Donohue *et al.,* 2010; Shepherd and Chapman, 1998; Toh *et al.*, 2008).

Influences of other nutrient alternates could facilitate seed germination like as soil conditions either with sufficient nutrients or its physical texture (Rivera-Aguilar *et al.*, 2005). Soil fertility remains important for seed germination, its further development (Venable and Lawlor, 1980). Seed germination response depends on available quantity and sources of supplements of essential plant nutrient element (Pérez-García and González-Benito, 2006).

Seed germination responses may quite be associated with the soil nutritional level. In this experiment various nutrient cultures are established for the cultivation of MMRI-Yellow var., of maize. Composition of each seed germination culture raised with local fruit based drinks (Table 1). A significantly differential pattern in seed germination as well as further growth of seedling among the treated to control cultures is observed. Maximum seed germination observed in control (T_0) and mixed juice treated cultures (T_6) as 93.33% and 86.66% respectively. It is decreased in the cultures supplemented with MAY - sparkling fruit juice and Murree's wheat bear either supplied in concentrated or diluted forms (*p<0.05*). Similar growth pattern after seed germination, seedling parts like as length of plumules and radicals even seedlings F. Wt. and its D. Wt. has been observed (Table 2).

Seed germination and its subsequent growth is impaired with various biochemical processes of plant metabolism, which consequently regulate their growth rate (Ashraf and Foolad, 2005; Kranner and Colville, 2011; Miransari and Smith, 2014).

The local drinks like as commercial fruit mixed juices, and beers even vines are based on different components of nutrients. In this way each juice could show different impact on plant or seed growth including its germination even it is best for plant callusing cultures (Haq *et al.*, 2011; Mgaya *et al.,* 2014; Nour *et al.*, 2012). It means that reduction or enhancement of plant biomass depends on availability of proper nutrition. While higher concentrations of juices may inhibit the germination or its further growth due to adverse effects of juices on degradation as well as mobilization of seed reserves (Djossa *et al.,* 2008; Evenari, 1949; Gardner *et al.,* 2000).

Morphological phenotypes of germinated seeds and its seedlings are expressions developed due to undergoing biochemical changes in the seed reserves. These are specific markers of seedlings growth and their development for which various enzymes of internal tissues are hydrolyzing seed reserve to convert complex cellular and organelle's molecules into simplest forms.

However, variations in rates of conversion of molecules into others depend on crops, species as well as external environmental conditions (Dürr *et al.,* 2015; Kakhki *et al.,* 2011; Kerrison *et al.*, 2015). Such as seed proteins are major component of seed reserve among the plant species, which are hydrolyzed with proteolytic enzymes at seed germination stage.

Proteins including other like as carbohydrates and lipids plays pivotal role being a source of nitrogen, carbon and energy for growing tissues. External nutritional sources could play a role in activation of hydrolytic enzymes, source of nutrition or even delay or inhibit the seed germination (Bragina *et al.*, 2003; Smykal *et al.*, 2014; Turner, 2010).

Table 2. Various morpho-biochemical attributes of maize (*Zea mays* L.) seedlings germinated under moisture conditions raised with local drinks (a 2-weeks culture).

S	Characteristics	T_0	T_1	T_2	T_3	T_4	T_5	T_6	*P*-sig.
a	Germination rate (%)	^{a}93.33±6.667	^{c}53.33±6.667	^{bc}13.33±6.667	^{d}26.66±6.667	^{d}26.66±6.667	^{bc}66.66±6.667	^{ab}86.66±6.667	***
b	Plumule length (cm)	^{b}7.333±0.273	^{d}4.233±0.203	^{c}5.100±0.115	^{b}2.833±0.088	^{ab}3.833±0.088	^{ab}5.367±0.145	^{ab}8.167±0.145	***
c	Radical length (cm)	^{a}8.400±0.173	^{c}5.733±0.233	^{b}6.900±0.173	^{e}3.800±0.173	^{d}4.667±0.145	^{b}7.167±0.145	^{a}8.833±0.088	***
d	Seedlings F.Wt (g)	^{a}9.400±0.095	^{e}5.004±0.099	^{d}5.403±0.123	^{f}4.814±0.039	^{e}4.813±0.090	^{c}8.921±0.090	^{a}9.889±0.061	***
e	Seedlings D.Wt (g)	^{a}1.440±0.026	^{c}0.890±0.009	^{b}1.022±0.033	^{e}0.687±0.008	^{d}0.782±0.009	^{b}0.991±0.009	^{a}1.501±0.008	***
f	Chlorophyll a (mg/g)	^{a}2.139±0.381	^{c}1.622±0.029	^{b}1.778±0.020	^{e}1.106±0.013	^{d}1.210±0.059	^{b}1.751±0.033	^{a}2.227±0.006	***
g	Chlorophyll b (mg/g)	^{a}0.994±0.006	^{c}0.770±0.009	^{b}0.801±0.007	^{d}0.547±0.005	^{c}0.749±0.007	^{b}0.814±0.008	^{a}1.009±0.010	***
h	Chlorophyll ab (mg/g)	^{b}3.133±0.043	^{d}2.393±0.023	^{c}2.577±0.013	^{f}1.653±0.011	^{e}1.959±0.053	^{c}2.565±0.026	^{a}3.236±0.005	***
i	Carotenoids (mg/g)	^{f}2.249±0.015	^{b}4.261±0.058	^{d}3.254±0.031	^{a}4.401±0.006	^{c}3.489±0.035	^{c}3.508±0.018	^{e}2.665±0.072	***
j	Total sugars (mg/ml)	^{ab}2.884±0.009	^{c}2.229±0.006	^{bc}2.571±0.012	^{d}1.782±0.329	^{c}2.347±0.014	^{c}2.442±0.011	^{c}2.975±0.006	***
k	R. sugars (mg/ml)	^{d}0.671±0.012	^{b}0.829±0.006	^{c}0.771±0.012	^{a}0.915±0.006	^{b}0.847±0.014	^{d}0.682±0.005	^{b}0.559±0.003	***
l	Total proteins (mg/ml)	^{a}3.717±0.041	^{d}2.471±0.012	^{c}2.529±0.006	^{f}2.047±0.014	^{e}2.113±0.013	^{d}2.435±0.003	^{b}2.765±0.017	***
m	Phenolics (mg/ml)	^{f}1.268±0.012	^{c}1.529±0.006	^{d}1.465±0.006	^{a}1.728±0.006	^{b}1.647±0.014	^{e}1.382±0.005	^{f}1.280±0.004	***
n	AOA (mmolTE/g F.Wt)	^{e}0.682±0.005	^{b}0.872±0.003	^{c}0.838±0.006	^{a}0.895±0.006	^{b}0.860±0.004	^{d}0.730±0.003	^{f}0.665±0.003	***

T_0: Distilled water control; T_1: MAY-sparkling fruit juice; T_2: ¼T_1 + distilled water; T_3: Murree's Wheat Beer; T_4: ¼T_3 + distilled water; T_5: Apple Mixed Juice: T_6: ¼T_5 + + distilled water; AOA: Antioxidants activity; R. sugars: Reducing sugars; *p-sig*: p significance; F.Wt. Fresh weight; D.Wt.: Dry weight.

Among the seed reserves, carbohydrates (exist as free sugar and polysaccharides-starch) are also being an important components available as essential energy rich sources for seed germination. Starch is degraded by α-*amylase* into soluble sugars after see germination (Bernal-Lugo Smykal Leopold, 1992; Hagely *et al.,* 2013; Hedge and Hofreiter, 1962). A differential pattern of total sugars observed among the seedlings maximum in T_0 and T_6 cultures, while minimum in T_3 and T_1 cultures. Similar pattern is also seen for total proteins, chlorophyll a and even overall total chlorophyll (ab) contents. Reduction in rate of starch degradation may be due to inhibition of hydrolytic enzymes lowered the seedlings growth rate. After seeds germination still rate of growth of seedling remained lowered in the T_3 and T_1 cultures, while observed maximum in T_0 and T_6 cultures. It might be declarable that after seed germination, seedlings are growing further under stress of supplied nutrients in the form of fruit juices. Stressed conditions are provable in the form of increase in reducing sugars, chlorophyll b, phenolics and antioxidants among the cultures (Table 2). Alleviation of these contents in seedling or plants is being their ability against applied stresses and remained at the same level until stress is passed over to normal conditions (Ali and Alqurainy, 2006; Michalak, 2006; Singh *et al.*, 2015). Anyhow initial nutrition have a key impact on seed germination, which is achievable with the optimization of initial nutrition condition for early seed growth of each crop.

Conclusions

Seed germination is an important step of early plant growth. Healthy seeds are germinated easily without delay than small sized or week seeds. After seedling stage, further growth potential depends on health of both typed seeds. Best vegetative as well as reproductive growth is achievable with supplementation of nutrients required for seed germination and its early growth. In this study, differential rates of seed germination and its further early seedling growth has observed among the maize var., MMRI-Yellow seeds of 2-weeks culture.

A similar and comparative seed germination and seedlings biomass attributes observed in control (distilled water) and mixed apple fruit juice (¼ dilution). Other seedlings (on alcoholic beer, non-alcoholic wine) showed reduction in plant growth rates because of imbalanced nutritional stress, which increases in osmoprotective among the cultures. Proper nutritional conditions at seed germination stage could be helpful to empower the seedlings for its further growth stages.

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