V.CHANDRA SEKHAR RAJU, *IJRRPAS*, 2011, Volume-1 Issue-4, Page-295-312.

ISSN:2249-1236

INTERNATIONAL JOURNAL OF RESEARCH AND REVIEWS IN PHARMACY AND APPLIED SCIENCES

Research Article

Received: 13-10-2011 *Accepted: 15-11-2011* *Published: 29-11-2011*

EVALUATION OF THE EFFECT OF OXYGEN SENSING ON SEED GERMINATION AND METABOLIC RATE IN FIVE DIFFERENT SEED VARIETIES

V. Chandra sekhar Raju, K. Babitha P. Anand

[1]M.tech Biotechnology, MIC College, Krishna district. [2]Lecturer in Biochemistry, MG College, Guntur.
[3]Lecturer in Biotechnology, Al College, Vijayawada.

ABSTRACT

At the center of every new plant growth cell is an atom of **carbon**, which the plant absorbs from carbon dioxide in the immediately surrounding air. As CO2 is used for its carbon, water vapor and Oxygen are released from the plant stomata as waste. If a plant is "breathing in" carbon dioxide and releasing oxygen as a waste product, what else could the plant possibly need the oxygen for? The short answer is **nutrient absorption**. No nutrient absorption occurs at the root zone unless **oxygen** is present. At a molecular level oxygen is required to transmit nutrients across the cell wall and into the roots. The effect of O2 partial pressure on the germination and the respiration of five cultivated species were studied. The reciprocal of the time necessary to observe rootlet emergence in 50% of the seeds was used to approach the germination rate. The maximum germination and respiration rates were reached in most seeds at O2 pressures close to that of air. Decreasing the O2 pressure produced a gradual decrease of the germination rate. Totally five plant seeds are tested for the oxygen sensing and germination rates. Then the metabolic rates of the corresponding seeds at germination were also tested by using the standard methods. The seeds could be classed in two groups according to their response to low O2 pressures.

1. Group I includes soya bean, pea and Horse gram:
2. Group II includes rice, wheat.

The germination rate of these seeds was gradually decreased by lowering the O2 partial pressure but germination still occurred, very slowly, at 0.1 kilopascal; the adenylate energy charge remained higher than 0.6. The metabolic rate of all these seeds are high during germination .at the of the germination the sugar, protein and lipid concentrations are very high at the ending of the of the germination these levels are very low

Key words: Molecular level oxygen, Nutrient absorption.

INTRODUCTION

Oxygen availability is a factor that often limits the desired cellular activities in biotechnological processes. This applies to plant cell cultures as well as to microbial cultures, despite the fact that plant cells have lower oxygen requirements than microorganisms. The large-scale cultivation of aerobic plant cells to high cell density is a challenging task. Plant cells are fragile and prone to hydrodynamic shear damage. Therefore, low mixing speeds are commonly used, which in turn leads to non-uniform mixing and low rates of mass transfer, including oxygen transfer. Oxygen availability is also considered to be limited in germinating seeds (Bewley and Black 1994). This is mainly a consequence of the covering layers of the seed coat acting as oxygen barriers. Seeds may also be subjected to oxygen deficiency in the soil, as a consequence of water lodging. The malting process exploits the germinative metabolism of cereal grains to produce malt, the main raw material of the brewing process. The process includes immersion of the kernels in water for several hours. During these so called steeping sessions, oxygen is quickly consumed by the kernel and the indigenous microbial community colonizing the kernel, leading to oxygen deprivation. Oxygen deficiency is therefore an important issue in industrial malting.

TYPES OF SEED DORMANCY AND THE ROLES OF ENVIRONMENTAL FACTORS

During seed maturation, the embryo enters a quiescent phase in response to desiccation. Seed germination can be defined as the resumption of growth of the embryo of the mature seed; it depends on the same environmental conditions as vegetative growth does. Water and oxygen must be available, the temperature must be suitable, and there must be no inhibitory substances present. However, in many cases a viable (living) seed will not germinate even though all the necessary environmental conditions for growth are satisfied. This phenomenon is termed **seed dormancy.** Seed dormancy introduces a temporal delay in the germination process that provides additional time for seed dispersal over greater geographical distances. It also maximizes seedling survival by preventing germination under unfavorable conditions.

Two types of seed dormancy have been recognized, coat-imposed dormancy and embryo dormancy.

1. **Coat-imposed dormancy**
2. **Embryo dormancy**

There are five basic mechanisms of coat-imposed dormancy:

1. *Prevention of water uptake.*
2. *Mechanical constraint.*
3. *Interference with gas exchange*
4. *Retention of inhibitors.*
5. *Inhibitor production.*

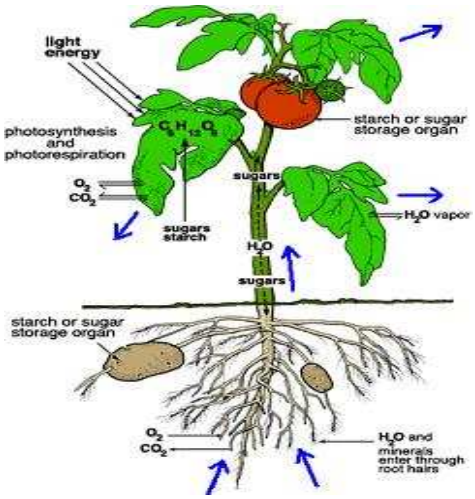


Fig 1: General metabolism of a plant

TYPES OF SEED DORMANCY AND THE ROLES OF ENVIRONMENTAL FACTORS

During seed maturation, the embryo enters a quiescent phase in response to desiccation. Seed germination can be defined as the resumption of growth of the embryo of the mature seed; it depends on the same environmental conditions as vegetative growth does. Water and oxygen must be available, the temperature must be suitable, and there must be no inhibitory substances present. However, in many cases a viable (living) seed will not germinate even though all the necessary environmental conditions for growth are satisfied. This phenomenon is termed **seed dormancy.** Seed dormancy introduces a temporal delay in the germination process that provides additional time for seed dispersal over greater geographical distances. It also maximizes seedling survival by preventing germination under unfavorable conditions.

Two types of seed dormancy have been recognized, coat-imposed dormancy and embryo dormancy.

3. **Coat-imposed dormancy**
4. **Embryo dormancy**

There are five basic mechanisms of coat-imposed dormancy:

6. *Prevention of water uptake.*
7. *Mechanical constraint.*
8. *Interference with gas exchange*
9. *Retention of inhibitors.*
10. *Inhibitor production.*

1. REVIEW OF LITERATURE

Seeds (caryopses or grains) of cereals from temperate climates often exhibit primary dormancy at harvest that is most evident at warm temperatures (>15 °C) (Corbineau and Côme, 1980; Lenoir *et al.*, 1983). Insufficient dormancy can result in pre-harvest sprouting in humid climates, while excessive dormancy can interfere with utilization of the grain for planting or malting (Benech-Arnold, 2004). The glumellae (lemma and palea) adhering to the caryopses, for example in barley (*Hordeum vulgare* L.) and oat (*Avena fatua* L.) grains, are primarily responsible for imposition of dormancy, as germination is much improved by their removal (Corbineau and Côme, 1980; Lenoir *et al.*, 1986; Benech-Arnold *et al.*, 1999). The dormancy present at harvest is lost during after-ripening (dry storage) as the temperature range for germination expands and germination occurs more rapidly and uniformly (Côme *et al.*, 1984).

It has been proposed that the effect of the glumellae on dormancy is to reduce the availability of oxygen (O_2) to the embryo (Pollock and Kirsop, 1956; Lenoir *et al.*, 1986; Lecat *et al.*, 1992). Fresh intact barley grains germinated poorly even in 100% O_2, while removal of the glumellae or after-ripening allowed complete germination to as low as 10% or 3% O_2, respectively (Lenoir *et al.*, 1986). However, the improvement in germination and lower O_2 requirement of naked grains following after-ripening suggests that factors in the embryo may also be involved. Abscisic acid (ABA) in particular plays a major role in regulating dormancy during

seed development (Feurtado and Kermode, 2007), and the ABA content of barley embryos was greater in a more dormant variety than in a less dormant variety (Benech-Arnold *et al.*, 1999).

EFFECTS OF ENVIRONMENTAL FACTORS ON GERMINATION

Major influences of temperature, salinity, and, to a lesser degree, light, oxygen, and sediment composition have been well documented for both terrestrial plants (Baskin and Baskin, 1998) and for many seagrass species (French and Moore, 2003; Orth et al., in press). Despite the important influences of environmental factors on seed germination, there has been little research in the affects of these parameters on many species of freshwater SAV, including *V. americana* (Barko et al, 1986).

Temperature 2. Light 3. Sediment Composition 4. Dissolved Oxygen 5. Salinity

OXYGEN UTILISATION IN SEED GERMINATION

The modern atmosphere contains approx.21 kPa oxygen. However, over the course of the past 550 million yr (Phanerozoic time), during which time the vascular plants invaded the land surface, plants have adapted to levels of atmospheric oxygen ranging from 13 to 51 kPa (Raven, 1991). This variation has been a major driver of plant evolution, and has led to the tuning of plant architecture/ultrastructure and metabolism to tolerate both low and high oxygen supply (Berner, 1999). Although over a shorter time-scale the atmospheric oxygen level may appear stable, plants must be able to adapt to variation in oxygen provision imposed by the local environment.

METABOLIC INDICATORS OF SEED HYPOXIA

There has long been a body of albeit indirect evidence for the existence of oxygen-depleted zones within the developing seed. For example, Boyle & Yeung (1983) noted the activity of alcohol dehydrogenases in bean (*Phaseolus vulgaris*) seeds, while Wager (1974b) observed the release of ethanol from pea seeds, and Gambhir *et al*. (1997) observed a decline in the cytosolic pH in the seeds of both soybean (*Glycine max*) and mustard (*Brassica juncea*) during certain stages of seed development. When soybean seeds were provided with oxygen, there was a shift in respiratory activity, along with a characteristic change in the ratio of adenine nucleotides (Gale, 1974; Shelp *et al*., 1995). The observation that both the number and unit size of the seed depend on the atmospheric oxygen level (*A. thaliana*, Kuang *et al*., 1998; rice (*Oryza sativa*), Akita & Tanaka, 1973; soybean, Quebedeaux & Hardy, 1975; wheat (*Triticum aestivum*), Musgrave & Strain, 1988) underlines the importance of hypoxia. Substantial oxygen concentrations in the atmosphere seem necessary to drive the diffusion of oxygen into the seed. Failing the establishment of these, the influx is too low to meet the demand, leading to abnormal seed development and even seed abortion.

Attempts have been made to model oxygen availability to the plant embryo (Collis-George & Melville, 1974; Dungey & Pinfield, 1980), but technical difficulties in obtaining an accurate measurement of localized concentrations of oxygen within the seed have hampered the development of predictive respiration and oxygen diffusion models.

GENE EXPRESSION AND METABOLISM UNDER LOW OXYGEN

Developing seeds are metabolically highly active. The early phase of development features meristematic growth, and the subsequent seed-filling phase is characterized by endoreduplication and the synthesis of storage products (Weber *et al*., 2005). A high respiration rate, combined with a limited gas exchange capability, results in oxygen depletion inside the seed. The question that remains is whether low oxygen really affects gene expression, mitochondrial respiration and overall (storage) metabolism. The oxygen levels commonly observed in seed tissue seem to be sufficient for mitochondrial cytochrome oxidase (COX) activity, as this enzyme has a low K_m (between 0.08–0.16 μm; Hoshi *et al*., 1993; Millar *et al*., 1994). However, there might be an intracellular oxygen gradient, thus the mean oxygen level measured by the micro sensor is likely not to be the value within the mitochondrion. Additionally, it is unclear whether these *in vitro* estimates of K_m apply *in vivo*. Thus, the internal steady-state oxygen concentration of 10 μm in rapeseed appears to be nonlimiting, but the oxygen-consuming respiratory flux may be affected.

OXYGEN AND ADH ACTIVITY

Oxygen is necessary for normal maintenance of the Krebs cycle and electron transport chain (Moore, *et al.*, 1998). When plentiful, oxygen serves as the ultimate electron acceptor in the electron transport chain, allowing for the regeneration of NAD+ from NADH. Under normoxic conditions, NAD+ is used by triose phosphatedehydrogenase to oxidize glyceraldehyde-3-phosphate to 1,3-bisphosphoglyceric acid. Under hypoxic or anoxic conditions, however, regeneration of NAD+ is not readily completed, leading to a breakdown of the glycolytic pathway (Moore, *et al.*, 1998). Other electron acceptors must, therefore, be used to regenerate NADH to NAD+ and fermentative metabolism is initiated. Rather than undergoing conversion to acetyl- CoA and entering into the Krebs cycle, pyruvic acid is converted via pyruvate decarboxylase to acetaldehyde. As a final step, acetaldehyde conversion into ethanol is catalyzed by alcohol dehydrogenase (ADH). During the catalysis, the hydrogen of NADH is removed and NAD+ is thus regenerated (Moore, *et al.*, 1998). Due to the fact that ADH activity correlates with relative amounts of root hypoxia, and its activity can be readily calculated by measuring the decrease of NADH in a spectrophotometric reaction, ADH serves as an ideal indicator of hypoxic stress (McKee and Mendelssohn, 1987).

AIM AND OBJECTIVE:

To determine the effect of O2 partial pressure on the germination and the respiration of five cultivated species. And to estimate the reciprocal of the time necessary to observe rootlet emergence in 50% of the seeds was used to approach the germination rate and metabolic rate in germinating seeds with the comparison of O2 content.

METERIALS AND METHODS

COLLECTION OF SEEDS

- Five different seed samples were collected from the crop fields
- Dry the seeds well and divide it into two groups

- Group I includes soya bean, pea and Horse gram.
- Group II includes rice, wheat.

These seed groups were sub divided into three types

- Type A: Un germinated
- Type B: Swollen (germination in presence of water)
- Type C: Germinated in soil

Fig 2: Five varieties of seed samples

PRETREATMENT OF SEEDS FOR STERILISATION

The seeds were decontaminated by treatment with Concentrated NaOCL and Sterile water for 30 min .The seeds were then placed on a layer of filter paper in Petri dishes containing a layer of glass beads (diameter 6 mm) Sprinkle enough water to moisten the filter paper.

DISSOLVED OXYGEN TESTING IN WATER

Carefully fill a 300-mL glass Biological Oxygen Demand (BOD) Stoppard bottle brim-full with sample water. Immediately add 2mL of manganese sulfate to the collection bottle by inserting the calibrated pipette just below the surface of the liquid. (If the reagent is added above the sample surface, you will introduce oxygen into the sample.) Squeeze the pipette slowly so no bubbles are introduced via the pipette. Add 2 mL of **alkali-iodide-azide** reagent in the same manner. Stopper the bottle with care to be sure no air is introduced. Mix the sample by inverting several times. Check for air bubbles; discard the sample and start over if any are seen. If

oxygen is present, a brownish-orange cloud of precipitate or floc will appear. When this floc has settle to the bottom, mix the sample by turning it upside down several times and let it settle again. Add 2 mL of concentrated sulfuric acid via a pipette held just above the surface of the sample. Carefully stopper and invert several times to dissolve the floc. At this point, the sample is "fixed" and can be stored for up to 8 hours if kept in a cool, dark place. As an added precaution, squirt distilled water along the stopper, and cap the bottle with aluminum foil and a rubber band during the storage period. In a glass flask, titrate 20 mL of the sample with sodium thiosulfate to a pale straw color. Titrate by slowly dropping titrant solution from a calibrated pipette into the flask and continually stirring or swirling the sample water. Add 2 mL of starch solution so a blue color forms. Continue slowly titrating until the sample turns clear. As this experiment reaches the endpoint, it will take only one drop of the titrant to eliminate the blue color. Be especially careful that each drop is fully mixed into the sample before adding the next. It is sometimes helpful to hold the flask up to a white sheet of paper to check for absence of the blue color.

SWOLLINATION OF SEEDS

Fill the beaker or Petri dish with water and place the seed samples into the water. Put it in the incubator

GERMINATION OF SEEDS

Fill a plastic dish with loamy soil (soil that is a mixture of sand, clay, and organic matter). The soil must have a high level of organic matter to help nourish the new seed varieties. Use a four inch by four inch plastic container to provide your seed with a safe place to germinate. The plastic container should be about five inches deep. Dig a hole into the soil. The hole should be at least a half an inch deep to accommodate your onion seeds. Use a pair of tweezers to lift the onion seeds and drop them into the holes you create. For the seeds are very small, move slowly and with caution to ensure you do not lose any seeds. Cover the seeds with soil. Sprinkle the water with approximate amount of oxygen. The uncovered Petri dishes were placed in 1 -L anaerobic jars; for each gas mixture, eight jars were connected in series. At the exit of the cylinder, the gas was sterilized through a Millipore membrane (0.2, um) and moistened before entry into the first jar. In order to limit the time of equilibration, the jars were submitted, three times, to a partial vacuum (about 30 mm Hg) and filled with the oxygen gas being studied. Afterwards, the seeds were left in the dark; a 100-ml/min gas stream was passed through the jars for the duration of the experimental period.After counting, the seeds were left under air to verify that germination could still occur. The criterion for germination was a 1-mm radicle emergence in dicot species and emergence of the radicle through the coleorhiza in the graminae. Fifty to 100 seeds were counted for each experimental point; results are the mean value for two or more experiments except for wheat

OXYGEN TEST PROCEDURE

Mix one volume of Sp. Gr.0.90 ammonium hydroxide with two volume of distilled water. Then add ammonium chloride until solid crystals are formed and settle at the bottom. Invert the reaction chamber set and fill it with copper wire in spiral form. Bring the copper wire in an upright position and connect with the burette.

Fill the reservoir and reaction chamber with test solution. Draw the test solution from the reservoir in the reaction chamber by turning the burette stop cock. Squeeze the rubber pipe to expel all the air and then close the stop cock, so that reaction chamber and the inter-connecting rubber pipe are completely filled with test solution. The purging vessel is half filled with water and the sample gas to be tested is allowed to bubble through water to atmosphere. Then controlling the flow of oxygen slowly opens the stop cock and allows the oxygen to pass into the burette. When burette is filled below the bottom mark, close the stop cock. Adjust the level of the gas in the burette to the 100 cc mark by holding the leveling bottom at the level of the liquid in the burette. Then carefully open both the cocks and allow the gas to bubble through the purging vessel to atmosphere. Pass the sample gas between burette and chamber several times. The oxygen in the sample reacts with the copper to form an oxide which dissolves in the solution. The unabsorbed impurities do not react with copper and are transferred from the chamber to the burette by lowering the leveling bottle. Observe all the unabsorbed materials giving out the percentage of impurities in the sample. This finally gives the percentage of oxygen in the sample indicating purity test of oxygen.

RELATIVE GERMINATION RATE

1. The time required to obtain 50% of germination, t5o, was determined by interpolation from the curve of germination percentage versus time, at each P02.
2. The t50 value corresponding to the maximum rate of germination, obtained in a nonlimiting p02, is t5o max and The t5o values at limiting P02 = x are t5O x.
3. The reciprocal of t5o was taken as the germination rate of the sample.

The relative germination rate at a P02 = x is given by

$$\text{Relative germination rate} = \frac{t50max}{t50x} X\ 100$$

NITRATE NITROGEN TEST

1. Weigh out 0.5 or 1.0 g. of dried ground plant material,
2. Add 100 ml of 2 percent acetic (V *IV)* to each sample and shake for 10 minutes on a platform or wrist action shaker and filter.
3. Two ml of these filtered extracts are diluted to 50 ml with distilled water.
4. Two ml of the diluted extracts are pipeted into test tubes.
5. The test tubes are placed in a container of water at room temperature (l8-28°C).
6. Eight ml of 2: I sulfuric acid-water (V/*V)* is added to each test tube.
7. The tubes may be removed from the water bath while adding the acid but must be returned to the water bath as soon as possible.
8. When the solutions have cooled, add 2 ml of 2, 6-dimethyl phenol in glacial acetic acid, (12.217 g per liter glacial acetic acid).

9. Stopper the tubes; shake each sample separately to mix and place back in Water immediately.
10. The percent transmission may be read at 325 nm 15 minutes after but no longer than 1 hour after mixing with the 2, 6dimethyl phenol reagent.
11. The solution must be kept in the water bath until read or the reaction will proceed un controlled and results will be erratic and high.
12. A reagent blank is carried through with each set of determinations.

EXTRACT PREPARATION FOR CARBOHYDRATE ESTIMATION

For soluble sugars and free amino acid determinations one gram of germinating seeds (cotyledons) were ground in a mortar and pestle in 5 mL of 80% ethanol (v/v).The mixture was boiled for 10 min and centrifuged at 2000 rpm for 10 min, The supernatant was collected and the pellet was re extracted in 5 mL of hot 80% ethanol. Supernatants from both extractions were combined. Total soluble sugar and reducing sugars were then determined by the phenol sulfuric acid (Dubois *et al*., 1956) and Nelson-Somogyi (Somogyi, 1952) methods, respectively, using glucose as standard. Free amino acids were determined in the final supernatant by ninhydrin method (Moore and Stein, 1954) using leucine as standard.

PHENOL SULPHURIC ACID METHOD FOR TOTAL CARBOHYDRATE

Follow the steps 1 to 4 as given in anthrone method for sample preparation. Pipette out 0.2, 0.4, 0.6, 0.8 and 1 mL of the working standard into a series of test tubes. Pipette out 0.1 and 0.2 mL of the sample solution in two separate test tubes. Make up the volume in each tube to 1 mL with water. Set a blank with 1 mL of water. Add 1 mL of phenol solution to each tube. Add 5 mL of 96% sulphuric acid to each tube and shake well. After 10 min shake the contents in the tubes and place in water bath at 25–30°C for 20 min. Read the colour at 490 nm. Calculate the amount of total carbohydrate present in the sample solution using the standard graph.

DETERMINATION OF REDUCING SUGARS BY NELSON-SOMOGYI METHOD

Weigh 100 mg of the sample and extract the sugars with hot 80% ethanol twice (5 mL each time). Collect the supernatant and evaporate it by keeping it on a water bath at 80°C. Add 10 mL water and dissolve the sugars. Pipette out aliquots of 0.1 or 0.2 mL to separate test tubes. Pipette out 0.2, 0.4, 0.6, 0.8 and 1 mL of the working standard solution into a series of test tubes. Make up the volume in both sample and standard tubes to 2 mL with distilled water. Pipette out 2 mL distilled water in a separate tube to set a blank. Add 1 mL of alkaline copper tartrate reagent to each tube. Place the tubes in boiling water for 10 minutes. Cool the tubes and add 1 mL of arsenomolybolic acid reagent to all the tubes. Make up the volume in each tube to 10 mL with water. Read the absorbance of blue colour at 620 nm after 10 min. From the graph drawn, calculate the amount of reducing sugars present in the sample.

EXTRACT PREPARATION FOR PROTEIN ESTIMATION

The sample was macerated in motor and pestle with 5 mL of phosphate buffer. Homogenate was centrifuged at 8000 rpm for 20 minutes. The supernatant was collected and extraction was repeated 4-5times. All the

supernatants was pooled and made the volume to 50 mL with phosphate buffer. One mL of above extract was taken and 1 mL of ice cold 20% TCA was added. The pellet was washed twice with acetone and again centrifuged at 8000 rpm. Supernatant was discarded and pellet was dissolved in 5 mL of 0.1 N NaOH. This was used for protein estimation. Total proteins were estimated by the method of Bradford using Ovalbumin as standard. Each experiment was repeated thrice

DETERMINATION OF PROTEINS BY BRADFORDS METHOD

1. Prepare/label 1.5 mL Eppendorf tubes for curve and sample replicates. You will need 3x7 tubes for the standard curve, and 5 tubes per sample. Pour out the approximate amount of Bradford reagent (obtainable from Biorad) that will be required into a clean beaker and cover it with Parafilm. It is better to overshoot a little than undershoot on the volume of Bradford reagent. Set the Bradford reagent aside and allow it to come to room temperature before using it.
2. Add nanopure water to the tubes as indicated in the table below. Add protein as indicated in the table below. Note that the standard curve is calculated on a BSA standard (Biorad Quickstart) at a concentration 2 mg/mL. Adjust accordingly for other concentrations. Proteins other than BSA may be used at the operator's discretion; however it is recommended that a commercial standard be used for improved accuracy and a more consistent batch. For the sample replicates more protein may be used for dilute samples, but the volume of water must be adjusted accordingly so that the total for vol. of water + vol. of sample = 800 uL.
3. Add the Bradford reagent as indicated below. Vortex the samples well and set them aside for 15 minutes to allow the samples to equilibrate. The total volume of each sample is 1 mL.
4. Pipette 200 uL of each sample into a clear 96-well plate. Use a different pipette tip for each tube. Scan on a plate reader at 595 nm.

EXTRACT PREPARATION AND LIPID ESTIMATION

Extraction and estimation of lipids was done by the method of Becker *et al.* (1978). One gram of germinated seeds was ground in mortar and pestle with chloroform-methanol mixture (2:1, v/v). For complete extraction the flask was kept at room temperature in the dark. Then chloroform and water (1:1, v/v) was added. The solution was subjected to centrifugation, three layers were observed. The methanol layer was discarded and lower organic layer was carefully collected and evaporated in water bath at 60°C. The weight of the lipid was determined. The results were expressed in terms of weight in mg of total lipids per gram of fresh tissue.

RESULTS AND DISCUSSION

The oxygen concentration of the seed germination was tested by the above procedures. The low oxygen content having seed will not easily germinate and the high concentrated seed takes less time for germination. The low internal oxygen concentration in the seed has a profound effect on storage product deposition, with clear implications for potential strategies to increase seed biomass and protein/oil yield of crops. The question is

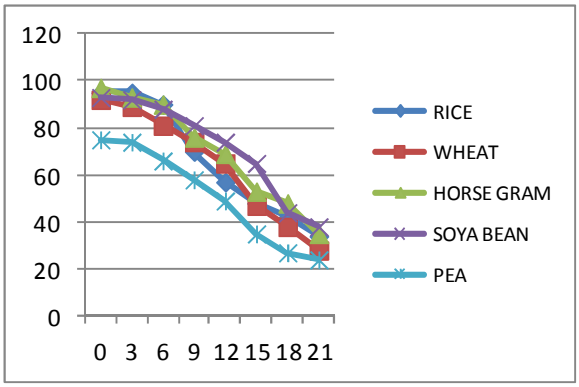
whether molecular approaches can be employed to increase oxygen availability within the crop seed. The most obvious strategy would be to promote the photosynthetic capacity of the seed.

1. Low internal oxygen levels in combination with high internal CO_2 levels (Wager, 1974a; Goffman *et al*., 2004) would minimize photorespiration. In such a way, the assimilate use efficiency is thought to increase.
2. Nature has generated cuticles and barriers, which may reduce oxygen uptake, but also help to prevent carbon dioxide loss. Carbon dioxide levels in seeds are high, so a low gas exchange capacity is helpful to promote the refixation of internally released carbon dioxide that would otherwise escape from the seed. Thus the carbon economy of seeds can be considerably improved (Schwender *et al*., 2004).
3. The cuticle acts as a barrier against water loss, pathogen invasion and UV penetration. Weakening the barrier may compromise some of these essential functions. The deposition of an intact cuticle may be a prerequisite for proper development (Pruitt *et al*., 2000).
4. Total lipid content reduced from 100 mg/gram to 49 mg/gram from 0 day to 21st day of germination. Approximately 50% reduction in lipid content was observed by the end of 15th day of germination. Less amount of reduction of lipids and triacylglycerols in the cotyledons indicated their lower level of mobilization and slower utilization by the embryonic axes. The liberated fatty acids might be used for the formation of membrane lipids in the growing embryos. Similar results were reported by Munshi *et al*. (2007) in sunflower seeds.

Oxygen content	**RICE (gm)**	**WHEAT (gm)**	**HORSE GRAM (gm)**	**SOYA BEAN (gm)**	**PEA (gm)**
0	75	64	77	80	63
20	67	62	65	76	57
30	54	57	55	72	47
50	45	52	49	55	43
80	31	45	30	48	34
100	28	32	29	42	21

Concentration of lipids V/S oxygen content

DAYS OF GERMINATION	RICE (gm)	WHEAT (gm)	HORSE GRAM (gm)	SOYA BEAN (gm)	PEA (gm)
0	95	92	97	93	75
3	95	89	93	92	74
6	90	81	90	88	66
9	70	74	76	81	58
12	57	65	69	74	49
15	48	47	53	65	35
18	42	38	48	44	27
21	34	28	35	38	24



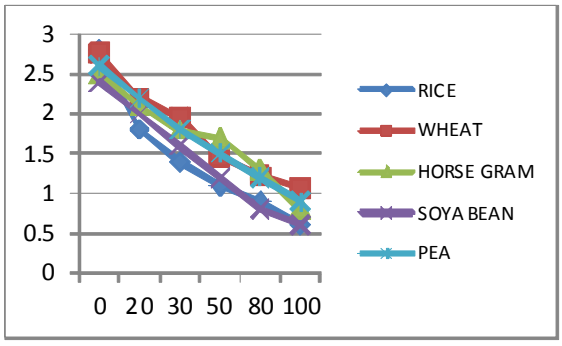
Concentration of lipids V/S days of germination

The total soluble sugars content varied from 1.70 mg/gram seeds to 0.8 mg/gram from 0 day to 21st day of germination. During germination, there was a decrease in storage carbohydrates and an increase in total soluble and reducing sugars up to 6th day of seed germination. This might be due to requirement of energy by growing plant at initial stages of seed germination. These results agree well with the results of Jaya and Venkataraman (1981) in chickpea and greengram and also by in white beans Kon *et al*. (1973).

AYS OF GERMINATION	RICE	WHEAT	HORSE GRAM	SOYA BEAN	PEA
0	1.9	1.96	1.87	1.9	2.1
3	2.1	2.01	1.98	1.99	2.18
6	2.3	2.34	2.22	2.21	2.24
9	1.8	2.2	2.1	2	2.2
12	1.4	1.96	1.8	1.6	1.8
15	1.1	1.45	1.7	1.2	1.5
18	0.9	1.22	1.3	0.8	1.2
21	0.6	1.06	0.8	0.6	0.9

Concentration of total carbohydrates V/S days of germination

Oxygen content	RICE	WHEAT	HORSE GRAM	SOYA BEAN	PEA
0	2.8	2.76	2.5	2.4	2.6
20	1.8	2.2	2.1	2	2.2
30	1.4	1.96	1.8	1.6	1.8
50	1.1	1.45	1.7	1.2	1.5
80	0.9	1.22	1.3	0.8	1.2
100	0.6	1.06	0.8	0.6	0.9



Concentration of total carbohydrates V/S oxygen content

Total reducing sugar levels were increased from 0 day (1.90 mg/gram) up to 6th day (2.86 mg/gram) of germination and thereafter reverse trend was observed. This might be due to mobilization and hydrolysis of seed polysaccharides during seed germination. Polysaccharides can be further hydrolysed by amylases which might be responsible for the increasing total reducing sugar levels in cotyledons during initial stages of seed germination (Bemfeld, 1962). Similar results were reported by Khetarpaul and Chauhan (1990) in rice.

DAYS OF GERMINATION	RICE	WHEAT	HORSE GRAM	SOYA BEAN	PEA
0	1.9	1.96	1.87	1.9	2.1
3	2.6	2.34	2.1	2	2.26
6	2.8	2.76	2.5	2.4	2.6
9	1.8	2.2	2.1	2	2.2
12	1.4	1.96	1.8	1.6	1.8
15	1.1	1.45	1.7	1.2	1.5
18	0.9	1.22	1.3	0.8	1.2
21	0.6	1.06	0.8	0.6	0.9

Concentration of total reducing carbohydrates V/S days of germination

Oxygen content	**RICE**	**WHEAT**	**HORSE GRAM**	**SOYA BEAN**	**PEA**
0	2.8	2.76	2.5	2.4	2.6
20	1.8	2.2	2.1	2	2.2

30	1.4	1.96	1.8	1.6	1.8
50	1.1	1.45	1.7	1.2	1.5
80	0.9	1.22	1.3	0.8	1.2
100	0.6	1.06	0.8	0.6	0.9

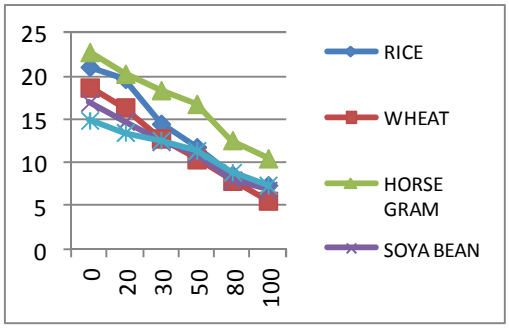
Concentration of total reducing carbohydrates V/S oxygen content

The total protein content decreased during seed germination in all seeds. The total protein content at the beginning of germination (0 day) was 24.5 mg/gram tissue and decreased to 12.6 mg/gram tissue at the end of 15th day of germination. There was a reduction in the protein content from day 0 to day 21, with a rapid decrease between 6th to 12th days of germination. The protein depletion in all seeds was very slow within the first 3 days of germination and rapid during the 6th and 21st days of germination coinciding with the hypocotyls extension. The loss of proteins from the cotyledons could be due to the transport of amino acids to the growing axes or to respiratory loss, or it might result in the accumulation of free amino acids in the cotyledons. These reports are similar to results of Beevers and Spittoesser (1968) in germinating Peas and in the cotyledons of Mung Bean Seedlings (Kern and Chrispeels, 1978).

DAYS OF GERMINATION	**RICE**	**WHEAT**	**HORSE GRAM**	**SOYA BEAN**	**PEA**
0	25.5	22.3	27.4	19.4	18.6
3	22.8	20.9	24.8	18.2	16.3
6	21.1	18.7	22.8	16.99	14.9
9	19.7	16.4	20.3	14.8	13.5
12	14.5	12.8	18.4	12.45	12.6
15	11.8	10.4	16.8	11	11.4
18	8.6	7.9	12.6	8	8.9
21	7.4	5.6	10.5	6.8	7.4

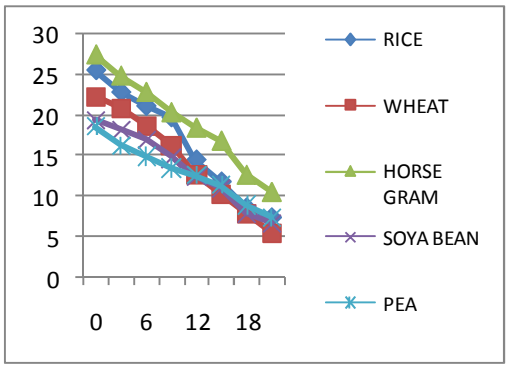
Concentration of total Proteins V/S days of germination

Oxygen content	RICE	WHEAT	HORSE GRAM	SOYA BEAN	PEA
0	21.1	18.7	22.8	16.99	14.9
20	19.7	16.4	20.3	14.8	13.5
30	14.5	12.8	18.4	12.45	12.6
50	11.8	10.4	16.8	11	11.4
80	8.6	7.9	12.6	8	8.9
100	7.4	5.6	10.5	6.8	7.4



Concentration of total Proteins V/S oxygen content

Overall, it may be advantageous for the seed to restrict its gas exchange. Even though the internal oxygen concentration falls to levels limiting respiration/storage metabolism, other considerations also come into play. It remains to be seen whether attempts to increase oxygen availability will result in higher seed biomass and storage capacity *in vivo*, or whether some or all of the above-mentioned factors act to negate any gain in crop productivity. A comprehensive understanding of the metabolism, structure and development of the seed is a prerequisite for initiating such approaches



CONCLUSION

Present study helps us to identify the relation between the oxygen, nitrogen, phosphorus, and potassium and seed germination.

- The oxygen is very important for the absorption and mobilization of the nutrients from the outer environment
- The atmospheric nitrogen is mixed with oxygen in the stage of seed germination and useful for the seed germination
- By comparing the results Major amount of oxygen is utilized by the nitrogen and lesser amount is used by the potassium
- So the internal oxygen is utilized by the nitrogen and phosphorus for the supplementation of the major nutrients of plant growth.
- To some extent it was used by the potassium for the mobilization of nutrients across the seed coat membranes.
- The seeds were rich in proteins, lipids as well as carbohydrates and their levels decreases as the germination progress, indicating their key role in the growth of embryonic axis. The work will be further continued to know the role different enzymes involved in germination of seeds and the purification of enzymes by different methods.

REFERENCES

1. Allen, R. J. L. [1940]. Biochem. J. 34, 858.
2. Berenblum, I. & Chain, E. [1938]. Biochem. J. 32, 286.
3. Bpdansky, A. [1932]. J. biol. Chem. 99, 197.
4. Fiske, C. H. & Subbarow, Y. [1925]. J. biol. Chem. 66, 375.
5. Kuttner, T. & Cohen, H. R. [1927]. J. biol. Chem. 75, 517.
6. Woods, J. T. & Mellon, M. G. [1941]. Industr. Engng Chem. (Anal. ed.), 13, 760.
7. Wu, H. [1920a. J. biol. Chem. 43, 189.
8. Zinzadz6, Ch. [1935]. Indu8tr. Engng Chem. (Anal. ed.), 7, 227.
9. Armstrong W, Brändle R, Jackson MB. Mechanisms of flood tolerance in plants. Acta Botanica Neerlandica. 1994;43:307–358.
10. Bailey-Serres J, Chang R. Sensing and signalling in response to oxygen deprivation in plants and other organisms. Annals of Botany. 2005;96:507–518.
11. Benamar A, Tallon C, Macherel D. Membrane integrity and oxidative properties of mitochondria isolated from imbibing pea seeds after priming or accelerated ageing. Seed Science Research. 2003;13:35–45.
12. Borisjuk L, Rolletschek H, Walenta S, Panitz R, Wobus U, Weber H. Energy status and its control on embryogenesis of legumes: ATP distribution within *Vicia faba* embryos is developmentally regulated and correlated with photosynthetic capacity. Plant Journal. 2003;36:318–329.

13. Botrel A, Magne C, Kaiser WM. Nitrate reduction, nitrite reduction and ammonium assimilation in barley roots in response to anoxia. Plant Physiology and Biochemistry. 1996;34:645–652.
14. Chang WW, Huang L, Shen M, Webster C, Burlingame AL, Roberts JK. Patterns of protein synthesis and tolerance of anoxia in root tips of maize seedlings acclimated to a low-oxygen environment, and identification of proteins by mass spectrometry. Plant Physiology. 2000; 122:295–318.
15. Conrath U, Amoroso G, Köhle H, Sültemeyer DF. Non-invasive online detection of nitric oxide from plants and some other organisms by mass spectrometry. Plant Journal. 2004; 38:1015–1022.