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Nitrate reductase activity of seven local corn cultivars from south west maluku district during water stress caused by polyethylene glycol 6000 under green house condition

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Abstract

Nitrate reductase activity is one of indicators that always observed in the correlation with the plant adaptation toward drought condition. One of procedures that could be conducted to develop the drought-tolerant of corn is by the selection of dryness by *in vitro* on limited scale like in the laboratory or in a green house, with using *polyethylene glycol* (PEG). This research objective is to know the activity of nitrate reductase on local corn cultivar from Kisar island South West Maluku regency after using *Polyethylene glycol* (PEG 6000) in a green house. The methods of this research consist of several steps such as preparation of corn seed, cultivation on growing media, drought stress treatment with 10% of polyethylene glycol, and analysis of Nitrate Reductase activity (NRA). The data collected were then analyzed descriptively. The result shows that on all local corn cultivars, the nitrate reductase activity on control group (without drought stress treatment) is lower than the corn suffering the drought stress by the treatment using PEG 6000 10%. It can be conclude that highest nitrate reductase activity value is obtained on the corn cultivar wich was treated with drought stress treatment using PEG 6000. The highest nitrate reductase activity is on *Kuning Genjah* cultivar (266.8 µmol/g), while the lowest one of nitrate reductase activity is on *Merah Delima Tongkol Putih* cultivar (81.36 µmol/g).

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Introduction

Corn or maize (*Zea mays* L.) is belongs to Gramineae or Poaceae, whose seeds can be used as a sources of carbohydrates. Globally, corn occupies the third position after rice and wheat as a food source of carbohydrates, whereas in Africa and Latin America, corn is in the first position as a source of carbohydrate, and in some developing countries corn is used as a staple food (Ahmad *et al.,* 2011).

In Indonesia, corn is also the second important food crop after rice (Alfons, 2005). In Maluku, the main corn production is in the southern isle, Kisar, and Wetar South West Maluku Regency. From the climate, the southern isle at South West Maluku regency is known by their dry season (areas with low rainfall). Susanto and Sirappa (2007), stated that the rainfall in South West Maluku regency, including Kisar island are about 991 - 1.102 mm/year. The rain intensity at Kisar island including 8 months of dry season, and 4 months of rain season. This low rainfall could have an influence to growth and development of cultivated plants including corn.

Even this corn is growing and reproduce at an area with limited rainfall, but corn in South West Maluku regency especially at Kisar island always cultivated on every cultivation season, and became the main food instead. This shows that corn at this area could be potentially cultivated on dry area and could be developed as superior corn cultivar.

Drought condition is one of abiotic factors that have an influence to growth, development, and the production of plants. The terminology of drought refers to a condition where plants lacking of water by the water deprivation in environment as the cultivation media (Tani *et al.,* 2019). According to Yadav and Sharma (2016) drought stress in plant caused by two kinds of condition, they are (1) the lacking of water supply in the root system area and (2) excessive water intake by leaf because of the increasing of evapotranspiration rate compare to the water absorption rate by the root, instead of the ground water condition still good. On dry soil, the drought stress caused by the water supply of the cultivation media are too small and could not fulfill the plant needs of water.

Drought stress have an influence to all aspect of plant growth and metabolism including the membrane integrity, pigment, osmotic balance, photosynthesis activity and also the production of plant (Anjum *et al.,*2011; Bhardwaj and Yadav, 2012). When plant deal with this dryness condition, plants have an adaptation mechanism to overcome this dryness condition so that it will not harming the plant itself. Plants have a mechanism to response the drought stress, they are by escape, avoidance, and tolerance (Akhtar and Nazir, 2013).

Plants tolerance toward the water deprivation condition could be done on biochemical level by the synthesis or degradation of proteins or several enzymes (Wang *et al.,* 2016) including Nitrate Reductase (Ananthi and Vijayaraghvan, 2012). Nitrate reductase activity is one of indicator that always observed in the correlation with the plant adaptation toward dryness condition, because nitrate reductase is one of key enzyme that contribute in nitrate reduction chain to be ammonium (Sepehr *et al.,*2012), and work in the amino acid synthesis, protein, chlorophyll and other substances containing nitrogen and the most important is in the vegetative and generative growth process of a plant. The biosynthesis of nitrate reductase depend on the availability of nitrogen and the activity could be induced with the availability of nitrate in leaf (Lawlor and Cornic, 2002).

Local cultivars in Kisar island South West Maluku regency seem to be adapted on that kind of growing condition with the limited water availability. This adaptation ability can be seen through several physiological, biochemical, and molecular variables such as the high content of proline and total soluble sugar as reported by Sinay and Karuwal (2014), high of proline content and yield components (Sinay *et al.,* 2015), and high levels of expression of drought resistant genes (Sinay and Arumingtyas, 2018). That's

why there should be a research to estimate whether the adaptation ability toward those water deprivation condition related to the activity of nitrate reductase.
One of procedures that could be conducted to develop the dry-tolerant corn is by the selection of dryness by in vitro on limited scale like in the laboratory or in a green house, with using *polyethylene glycol* (PEG) (Sofo and Shokoofeh, 2018). Polyethylene glycol (PEG) is one of substance that could decrease the osmotic potential of ground water by the matrix activity of ethylene oxide sub-unit that could bond with water molecule forming a hydrogen bond.

Besides that, the activity measurement of nitrate reductase by the stimulation of drought stress in green house, function to inform the adaptation ability of local corn cultivar from Kisar island that could be used as gene source to form a dry-tolerant cultivar. According to (Monneveux *et al.,* 2006) the supreme-cultivar selection could be determined by the availability of germ plasm biodiversity, that is the local cultivar or landrace, that function to provide the genetic material that it is needed to assembly the superior varieties with an endure character that could survive in dryness condition.

This research objective is to know the activity of nitrate reductase on local corn cultivar from Kisar island South West Maluku regency after using *Polyethylene glycol* (PEG 6000) in a green house.

Materials and methods

Preparation of corn seedling

Corn seed for this research obtained from the local corn farmer at Kisar island of South West Maluku regency. Corn seed for seedling soaked with water for about 24 hours.

The seed then put on a *polybag* 12 × 5 cm in size with 1 piece of seed/polybag, and allowed to grow until seven days after seedling.

Cultivate on Growing media

Corn seedling than transplanted in to growing media on polybag size 30 × 20 cm. and 300 mL of water was given every day.

PEG 10% solution preparation, and the Drought stress

PEG 10% (w/v) volume 300 mL made by weigh out PEG 6000 as much as 30 mg and mixed with 100 mL of aquadest, and the remaining aquadest was added to reach a volume of 300 mL. Treatment using PEG 6000 10% was carried out when the seedlings were seven days after the cultivation (14 days after seedling) every day until 30 days after cultivation (23 day after seedling). The volume of PEG volume was given is equal to the volume of water as much as 300 mL/polybag. On control group, there is only water is given with the same volume to the PEG treatment as much as 300 mL/polybag.

Analysis of Nitrate Reductase Activity (NRA) Level

Leaf Sample withdrawal for NRA Analysis

Fresh leaves (2nd leaf from the tip) washed with tap water, then separated between leaf vein and the leaf blade, then prepared for NRA level analysis.

Measurement of NRA Level

The measurement of Nitrate reductase activity (NRA) level was carried out following the method from Li *et al.* (2014), Krywult and Bielec (2014), and Kim and Seo (2018) with a little modification. Leaf blade was cut and weighed out about 0.5 grams. Pieces of leaves then put into 5 mL of phosphate buffer solution in a dark tube, and soaked for about 24 hours. After 24 hours, buffer solution then removed, and replaced with the new one (Phosphate Buffer made from NaH_2PO_4 0.1M and Na_2HPO_4 0.1 M). Then 0.1 mL of $NaNO_3$ was added with a micro-pipette and the time was counted as the incubation time begin for two hours. While waiting for the incubation for almost 2 hours, the colorant reagent was prepared by mixing the 0.2 ml of N-Naphthylenediamine solution 0.02% (w/v) and 0.2 mL of 1% sulphanilamide (w/v) in 3N of Hydrogen Cloride. After incubated for two hours, the 0.1 mL of incubated solution from the dark tube then take and pour into the reaction tube containing colorant reagent, and wait until the color changes into pink as a sign that the nitrate reduction occurred and

nitrate changes into nitrite by the nitrate reductase enzyme. One of the reaction tube will not be patched with filtrate and being used as Blanco. After the color was changed then the 2.5 mL of aquadest was added, then moved in to the cabinet for the absorbance measurement with using UV spectrophotometer on a wavelength of 540 nm.

The Nitrate reductase activity (NRA) level was expressed in a unit of micromole nitrate/gram of fresh sample (Garg and Singla, 2005), with using formulas as follows:

$$\text{NRA}\,(\mu\text{mol/gr}) = \frac{\text{Sample Absorbance}}{\text{Standard Absorbance}} \times 50 \times \frac{100}{\text{FW}} \times \frac{1}{\text{T}} \times \frac{1}{1000}$$

Research result data is the average of measurement result of nitrate reductase activity (NRA) from two times of replication. The data analysis technique will be analyzed descriptively and served in form of pictures.

Result

The research result shows that on all local corn cultivar, the NRA on control group (without drought stress treatment) is lower than the corn suffering the drought stress by the treatment using PEG 6000 10% (Fig. 1). This shows that the NRA value was increased when the plants was faced to the drought stress.

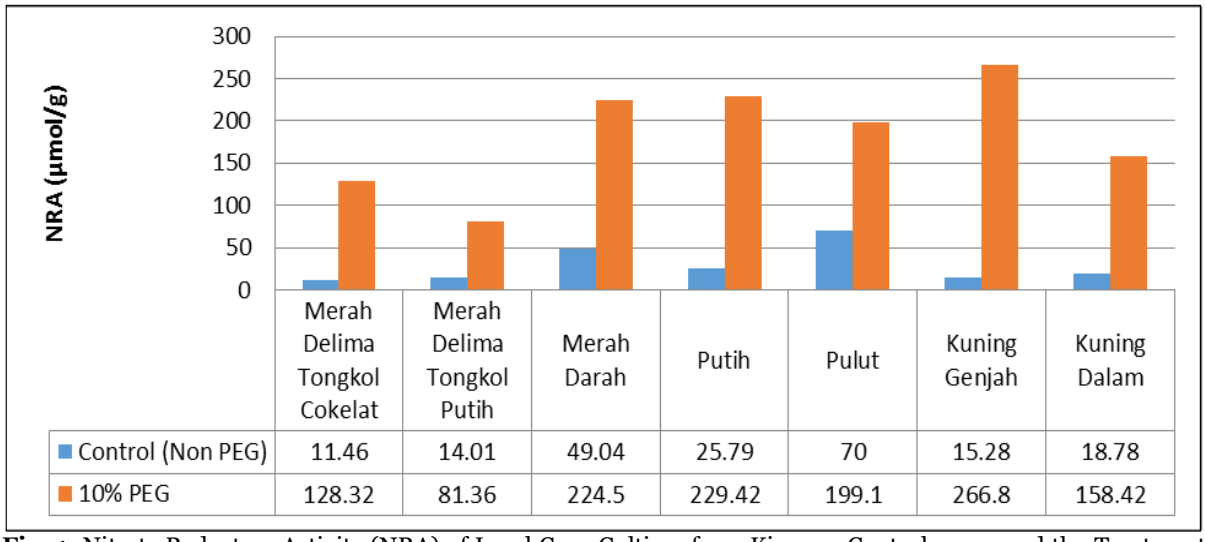

Fig. 1. Nitrate Reductase Activity (NRA) of Local Corn Cultivar from Kisar on Control group and the Treatment group using PEG 10%.

Discussion

According to the figure 1, it can be seen that each cultivar shows different nitrate reductase activity. When compared with control, the highest value of NRA was shown by all of corn cultivars which was triggered with 10% of polyethilene glycol 6000. For those corn cultivars with 10% of PEG treatment, the highest NRA value was obtained on *Kuning Genjah* cultivar (266.8 µmol/g), while the lowest NRA value was obtained on *Merah Delima Tongkol Putih* cultivar (81.36 µmol/g).

For the control, the highset NRA value was obtained on the *Merah Darah* cultivar, and the lowest one was obtained on the *Merah Delima Tongkol Cokelat* cultivar. From the above figure also can be seen that the cultivar with highest or the lowest value of NRA level on control were not the highest or the lowest in the PEG treatment. This means that each cultivar has different ways in response to water availability on their growing media, both on the control nor the PEG treatment.

Some results of current investigation revealed that there were a decrease in nitrate reductase activity on some plant along with the dryness, and this has been reported in cowpea (Silveira *et al.,* 2001), sun flower (Correia *et al.,* 2005), wheat (Fresneau *et al.,* 2007),

and cotton (Ananthi and Vijayaraghavan, 2012). These report is in line with Bian *et al.,* (2017) who was stated that on dryness condition, a lot of biochemical and molecular activity occurred in plant such as the inhibition and degradation of several protein. Fresneau *et al.,* (2007) explained that during drought stress, the supply of ground water decreased, so the absorption ability of water by plant will also decreased. This condition can cause a decrease of nitrogen uptake from ground and could affects the decreasing of metabolism rate as nitrogen playing role in chlorophyll biosynthesis, the decreasing of photosynthesis rate, and the decreasing of protein synthesis rate.

Contrary to what was previously reported by many researchers that the level of NRA actually decreases when drought stress occurs, the result of our current study especially for the corn from Kisar Island actually show that cultivars with PEG treatment has highest NRA value than the control. This means that drought stress which was caused by PEG can lead the increasing in NRA level especially for those local corn cultivars.

This shows that even though they grows in drought condition, the local cultivar from Kisar island could grow with no problem instead and on their metabolism including nitrate reductase activity. This also means that local corn cultivars from Kisar island have an great adaptive ability toward the drought stress condition. With the increasing of nitrate reductase in the plant with drought condition, it will stimulate the increasing of protein synthesis, chlorophyll production, nucleic acid, amino acid and other substituent needed by plant for vegetative and generative development.

Nitrate reductase is one of intracellular enzymes that is highly sensitive towards the water stress (Silveira *et al.,* 2001). This enzyme function is to reduce the nitrate ion (NO_3) into nitrite ion (NO_2). Some plants taking nitrogen from soil in form of ammonium (NH_4^+) or nitrate (NO_3^-), nitrate is one of ion that mostly absorbed by plants. Absorbed nitrate then reduced by Nitrate Reductase enzyme to turn it into nitrite that then reduced further to be ammonium. Ammonium then will be collected with all photosynthesis final products by the glutamine biosynthesis and glutamate to form amino acid, protein, chlorophyll and other substituents containing nitrogen. By the transcription and translation process, those amino acids then forming protein. The protein then function as functional protein and or structural protein. Protein will give influence to growth and development of plant, and also forming a biomass, and determine the productivity rate.

Nitrate reductase activity could be used as selection criteria for plant on high production priority on breading plant program (Fahad *et al.,* 2017). This means that the plant with high reductase activity on environment with drought stress condition is potentially developed as drought-tolerant plant, because the ability to form the protein as enzyme such as nitrate reductase on drought condition, so this shows that those plants could survive although it grows in insufficient environment condition, and could be recommended to cultivate those corn especially as the cultivated plant in drought prone area.

Conclusion

Based on the research result, it can be conclude that Highest nitrate reductase activity value is on the corn given with drought stress treatment using PEG 6000 under green house.The highest nitrate reductase activity is on *Kuning Genjah* cultivar (266.8µmol/g), while the lowest nitrate reductase activity is on *Merah DelimaTongkol Putih* cultivar (81.36µmol/g).

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