Effect of various polyethylene glycol concentrations on the growth of seedlings of *Indigofera zollingeriana*

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**Abstract.** The use of dry land for the cultivation of *Indigofera zollingeriana* is an effort to develop forages in the dry season, especially on the land lacked of water. The study was aimed to determine the effect of various concentrations of polyethylene glycol (PEG) 6000 stress on *I. zollingeriana* seeds by evaluation of drought tolerance test from germination to nursery phase. The study was compiled based on a randomized block design (RBD) with five treatments and three groups. The treatment groups were as follow: P1=water without PEG (control), P2=water+5% PEG (10 g/liter of water), P3=water+10% PEG (20 g/liter of water), P4=water+15% PEG (30 g/liter of water), and P5=water+20% PEG (40 g/liter of water). The parameters observed in this study were the root length, growth percentage, and leaf area index. Results showed that the seeds of *I. zollingeriana* were able to adapt to drought at the PEG concentration of 20%, despite the salt stress which affected the plant growth.

1. **Introduction**

The area of dry land in Indonesia was 148, million ha and only about 76.22 million ha was used as productive dry land, approximately [1]. Dryland is prone to erosion and is nutrient-poor, thus soil and water conservation efforts are needed. One type of legume plants able to adapt to drought conditions and being cover crops is *Indigofera zollingeriana*. This crop was tolerant of standing water and was able to survive and produce at the level of severe drought stress (25% field capacity), even though it experiences a decrease in productivity[2,3]. The adaptation ability of *Indigofera zollingeriana* on NaCl medium showed that this plant was also very tolerant of high salinity (9.9 ds/s), despite the effects of stress which affected plant growth [4].

*Indigofera zollingeriana* was able to grow in drought stress conditions, had a high production, high crude protein, higher Ca, and low crude fiber content compared to other legumes [5]. The nutritional content of *I. zollingeriana* contains 27.9% crude protein, 15.25% crude fiber, 0.22% calcium, and 0.18% phosphorus. Because of its resistance to drought, *I. zollingeriana* was usually developed in tropical climates to meet feed needs, especially in the dry season. Nevertheless, research was needed on the tolerance level of *I. zollingeriana* to drought. The test could use polyethylene glycol (PEG) substances. Polyethylene glycol (PEG) was used to control seed imbibition and hydration. This substance was also used to test seed resistance to drought by the drought index determination [6–8]. Sofinoris [9] suggested that concentration and immersion time in Polyethylene Glycol 6000 (PEG-6000) types affect the viabilty of cotton (*Gossypium hirsutum* L) seeds.
Research using PEG could also be done to determine the tolerance level of drought stress in *I. zollingeriana* plants. This research was aimed to determine the effect of various concentrations of PEG-6000 stress on seeds for drought resistance from germination to nursery phases. It was expected to provide information for the development of feed crops on dry land.

2. Methods
Indigofera seeds were germinated on cotton media to stimulate germination. Then transferred to a nursery tray containing sand, compost, and soil with a ratio of 1:1:1. Seeds that had formed leaves were then transferred to polybags that contained sand, soil, and compost and arranged according to the treatment groups. Each polybag was inserted into a plastic bottle that filled with a mixture of water and PEG according to the treatment groups as follow: P1=water without PEG (control), P2=water+5% PEG (10g/liter of water), P3=water+10% PEG (20g/liter of water), P4=water+15% PEG (30g/liter of water), and P5=water+20% PEG (40 g/liter of water).

The study was compiled based on a randomized block design (RBD) with five treatments and three groups so that there were 15 observation units. The parameters observed in this study were: growth percentage, number of leaves, plant height, leaf area index, root length and amount of chlorophyll.

3. Results and discussion
The effect of various concentrations of PEG on plants could be determined from several parameters. The effect of different level of PEG-6000 on leaf number, plant height, leaf area index and growth ability of *I. zollingeriana* were presented in table 1.

| Treatment | Parameters |  |
|-----------|------------|---|
|           | Root length (cm) | Leaf area index (cm) | Plant resistance (%) |
| P1        | 29.00±8.71\(^a\) | 27.20±18.22 | 23.3±0.57 |
| P2        | 12.33±3.05\(^b\) | 5.56±0.40 | 23.3±0.57 |
| P3        | 10.66±5.50\(^b\) | 10.46±11.31 | 23.3±0.57 |
| P4        | 7.33±1.52\(^b\) | 11.34±5.98 | 20.0±0.00 |
| P5        | 6.00±2.00\(^b\) | 1.90±1.94 | 13.3±0.57 |

\(^{ab}\)Superscript with different notation letter on the same column showed a highly significant difference (P<0.05). P1=water without PEG (control), P2=water+5% PEG (10g/liter of water), P3=water+10% PEG (20g/liter of water), P4=water+15% PEG (30g/liter of water), and P5=water+20% PEG (40 g/liter of water).

Analysis of variance showed that the PEG concentration had a significant effect (P<0.05) on root length. The DMRT test showed that the average root length of the plant in P1 was higher than treatment P2, P3, P4, and P5, respectively. Table 1 showed that the higher the concentration of PEG caused inhibition to the development of roots. Suggested that plants that had a ratio of root lengths at a lack of water condition, indicated that the plant was resistant to water shortages and was a potential root morphological character as an indicator of lack of water in plants [10].

Indigofera seeds that were not treated with PEG showed longer roots than those treated with PEG for the drought stress test. Plant roots in the control treatment (P1) were longer because they look for water availability in the environment. PEG was functioned as a stimulant for drought stress so that plants that were treated with PEG had shorter roots because the plant roots were gripped by the presence of PEG content. This was consistent with the opinion of [11] when drought stress occurred, plants developed more root systems. Root cells undergo changes, by increasing or reducing the number and size to adapt to drought stress. The physiological and morphology responses of soybean plants that were resistant to drought stress were the increase in root dry weight and root length,
increase in proline content and decrease in leaf osmotic potential [12–14] and an increase in root dry weight in peanuts [15,16]. In addition, an increase in the number of root hairs would increase the ability of plants to absorb water [17].

The administration of PEG solution had no significant effect (P> 0.05) on the durability of the I. zollingeriana plants. Table 1 showed the same plant tolerance on P1, P2, and P3, which was 23.3%, but a lower percentage on P4 and P5, 20% and 15%, respectively. PEG in plants could inhibit the absorption of water from the environment, thus inhibited the optimal process of plant growth and development, and decreased production. Indigofera zollingeriana plants with 10% PEG concentrations had the same durability as control plants (without PEG). The level of tolerance of plants was highly dependent on how plants adapt and respond to various types of stress, especially drought, physiologically and morphologically. Dryness was the main factor that limits growth and development in higher plants because the drought was a common occurrence in many environments and many annual plant species had developed mechanisms to overcome limited water availability [18].

According to [19], plants that experienced drought stress would try to make physiological changes as a form of adaptation in order to survive. One of which adaptation was the ability of plants to maintain turgor pressure or osmotic adjustment. Cells capable of making osmotic adjustments under stressed conditions were believed to be variants that carried tolerance to drought stress [20]. The ability of PEG as reported by [21] in a study of potato selection methods responded to drought stress showed that PEG was able to hold water so that it was not available to plants. The osmotic stress applied to growth media caused the water supply needed by plants in the root area insufficient for evapotranspiration. This imbalance caused plants to experience drought stress. Therefore, plants would experience adaptation mechanisms such as changes in plant physiology and morphology.

The administration of PEG solution had no effect (P> 0.05) on the leaf area index of I. zollingeriana plants. Table 1 showed that the largest leaf area index was obtained in P1 (control) with the value of 27.20 cm and the lowest in treatment P5 with the value of 1.90 cm, while in treatment P2, P3 and P4 had the same leaf area index. This was due to the significant decrease in leaf area in drought conditions was one of the mechanisms of morphological adjustment to reduce water loss through transpiration, so that the leaves, especially in the young leaves were not damaged. According to [22], the response of plants to water stress was different depending on the severity of water stress experienced, the type of plant and the age of the plant. But in general, the occurrence of water stress would affect the size of plant organs, one of which was narrowing and shortening of leaves. Reduced leaf area was normal in plants that experience water stress.

Palupi and Dedywiryanto [23] suggested several ways to respond to water shortages in plants were by closing leaf stomata to reduce transpiration, then followed by a decrease in metabolism [24], decreased in plant growth, and developed a root system. The decreased in leaf area growth to drought conditions was caused by a decrease in leaf cell turgor pressure due to a decrease in leaf water content. This caused the inhibition of CO2 absorption by stomata and non-stomata, so that the rate of photosynthesis decreased [25]. The decreased in leaf area growth due to drought would reduce the rate of photosynthesis, but the mechanism was not always detrimental since there was a genotype of rice which could still produce in that condition [26].

4. Conclusion
Indigofera zollingeriana seedlings were able to adapt to drought tolerance at the PEG concentration of 20%, despite the salt stress which affected the plant growth.

References
[1] Hidayat A and Mulyani A 2002 Lahan kering untuk pertanian Teknologi Pengolahan Lahan Kering menuju Pertanian Produktif dan Ramah Lingkungan. Bogor (ID): Pusat Penelitian Tanah dan Agroklimat pp 1–39
[2] Hassen A, Retman N F G, Van Niekerk W A and Tjelele T J 2007 Influence of season/year and species on chemical composition and in vitro digestibility of five Indigofera accessions Anim. Feed Sci. Technol. 136 312–22
[3] Herdiawan I 2013 Pertumbuhan tanaman pakan ternak leguminosa pohon Indigofera zollingeriana pada berbagai taraf perlakuan cekaman kekeringan JITV 18 258–64
[4] Nadir M, Anugrah M J and Khaerani P I 2018 Salt salinity tolerance on nursery of Indigofera zollingeriana IOP Conf. Ser. Earth Environ. Sci. 156 12027
[5] van Man N, van Hao N and minh Tri V 1995 Biomass production of some leguminous shrubs and trees in Vietnam Livest. Res. Rural Dev. 7 1–5
[6] Nemoto K, Morita S and Baba T 1995 Shoot and root development in rice related to the phyllochron Crop Sci. 35 24–9
[7] Bouslama M and Schapaugh W T 1984 Stress tolerance in soybeans. I. Evaluation of three screening techniques for heat and drought tolerance 1 Crop Sci. 24 933–7
[8] McDonald M B, Vertucci C W and Roos E E 1988 Soybean seed imbibition: water absorption by seed parts Crop Sci. 28 993–7
[9] Sofinoris S 2009 Penelitian viabilitas (priming) benih kapas (Gossypium hirsutum L.) dengan Polyethylene Glycol (PEG) 6000 (Malang: Universitas Islam Maulana Malik Ibrahim)
[10] Nio S A and Torey P 2013 Karakter morfologi akar sebagai indikator kekurangan air pada tanaman Bioslokos 3 31–9
[11] Lynch J P and Brown K M 2012 New roots for agriculture: exploiting the root phenotype Philos. Trans. R. Soc. B Biol. Sci. 367 1598–604
[12] Hamim S D and Jusuf M 1996 Beberapa karakteristik morfologi dan fisiologi kedelai toleran dan peka terhadap cekaman kekeringan Hayati 3 30–4
[13] Ashri K 2006 Akumulasi Enzim Antioksidan dan Proline pada Beberapa Varietas Kedelai Toleran dan Peka Cekaman Kekeringan Thesis (Bogor: Institut Pertanian Bogor)
[14] Lobato A K S, Santos Filho B G, Costa R C L, Oliveira Neto C E, Meirelles A C S, Cruz F J R, Alves G A R, Neves H K B, Pita J D and Lopes M J S 2008 Physiological and biochemical changes in soybean (Glycine max) plants under progressive water deficit during the vegetative phase Agric. J. 3 327–33
[15] Puangbut D, Jogloy S, Vorasoot N, Akkasaeng C, Kesmala T, Rachaputi R C N, Wright G C and Patanothai A 2009 Association of root dry weight and transpiration efficiency of peanut genotypes under early season drought Agric. Water Manag. 96 1460–6
[16] Songsri P, Jogloy S, Holbrook C C, Kesmala T, Vorasoot N, Akkasaeng C and Patanothai A 2009 Association of root, specific leaf area and SPAD chlorophyll meter reading to water use efficiency of peanut under different available soil water Agric. water Manag. 96 790–8
[17] Vasellati V, Oesterheld M, Medan D and Loreti J 2001 Effects of flooding and drought on the anatomy of Paspalum dilatatum Ann. Bot. 88 355–60
[18] Zhang M W, Zhang R F, Zhang F X and Liu R H 2010 Phenolic profiles and antioxidant activity of black rice bran of different commercially available varieties J. Agric. Food Chem. 58 7580–7
[19] Khaerana K, Ghulamahdi M and Purwakusumah E D 2008 Pengaruh cekaman kekeringan dan umur panen terhadap pertumbuhan dan kandungan xanthorrhizol temulawak (Curcuma xanthorrhiza roxb.) Indones. J. Agron. 36
[20] Sutjahjo S H, Kadir A and Mariska I 2007 Efektivitas Polietilen Glikol sebagai Bahan Penyeleksi Karakter Nila yang Diiradiasi Sinar Gamma untuk Toleransi Terhadap Cekaman Kekeringan J. Ilmu Pertan. Indonesia 9 48–57
[21] Charloq C, Panjaitan E and Sirait B A 2012 Study of early screening of potato (Solanum tuberosum L.) as a result of drought stress (in vitro) 2nd Syiah Kuala University Annual International Conference 2012 (Syiah Kuala University)
[22] Soedarsono 1997 Respon fisiologi tanaman kakao terhadap cekaman air War. Puslit Kopi dan Kakao 13 96–109
[23] Palupi E R and Dedywiryanto Y 2008 Kajian karakter ketahanan terhadap cekaman kekeringan pada beberapa genotipe bibit kelapa sawit (Elaeis guineensis Jacq.) J. Agron. Indonesia. (Indonesian J. Agron. 36
[24] Flexas J and Medrano H 2002 Drought-inhibition of photosynthesis in C3 plants: stomatal and non-stomatal limitations revisited Ann. Bot. 89 183–9
[25] Barlow E W R and Boersma L 1976 Interaction between leaf elongation, photosynthesis, and carbohydrate levels of water-stressed corn seedlings J. Agron. 68 923–6
[26] Sastrowinoto S 1985 Kajian Gaya Cabut sebagai Metode Penyaringan Ketahanan terhadap Kekeringan dan Genetika Perakaran Padi Lahan Kering (Yogyakarta: Universitas Gadjah Mada)