The Role of Micropyle for Seed Germination at Grass Pea

Edi Wiraguna [a,b,c*]

[a]Technology and Management of Plantation Production, College of Vocational Studies, IPB University, Bogor, Indonesia
[b]Production Technology and Development of Agricultural Communities, College of Vocational Studies, IPB University
[c]Center for Agrarian Studies, IPB University Bogor, Indonesia

*Corresponding author. Email: ediwiraguna@apps.ipb.ac.id
Phone: +62251 8376845
Postal code: 16128

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Abstract:

Grass pea is one of important legumes for human consumption and is reputed to be tolerant to abiotic stresses such as waterlogging at germination. However, the role of micropyle for seed germination has not been studied. First, micropyle was identified by cutting the grass pea seed in half and observing the seeds under electron microscopy. Second, the location of micropyle was identified nearby hilum, similar to soybean seeds. Second, the micropyle was covered with lanolin to block water imbibition. The rate of imbibition and germination was then observed. Lanolin significantly reduced water imbibition. The micropyle covered by lanolin had a lower germination percentage at lower than 57% than those uncovered by lanolin at 87%. Lastly, the micropyle sizes of various grass pea genotypes were identified by capturing seed images under light microscopy and converting the sizes to mm using computer software (ImageJ). However, there is no correlation between micropyle size and seed weight. These findings add information on the location and role of micropyle for grass pea seed germination.

Keywords: micropyle; grass pea; seeds; germination

Introduction:

Grass pea (*Lathyrus sativus* L.) is one of important legume crops in Bangladesh, Ethiopia and Pakistan (Benková and Zakova, 2001; Campbell *et al.*, 1994; Tadesse and Bekele, 2001; Yigzaw *et al.*, 2001). Grass pea contains high protein in seeds higher than chickpea (*Cicer arietinum* L., ~18%) and similar to that of field pea (*Pisum sativum* L.) and faba bean (*Vicia faba* L.) (Yan *et al.*, 2006). Grass pea is also reputed to be tolerant to abiotic stresses such as flooding and low soil fertility (Benková and Zakova, 2001; Campbell *et al.*, 1994; Tadesse and Bekele, 2001; Yigzaw *et al.*, 2001).

Micropyle is a small pore in the ripe see used as an entry of solute into seeds to promote germination (Manohar and Heydecker, 1964; Munz *et al*., 2017). In *Rhus sp.* and *Geranium carolinianum*, micropyle is located nearby hilum and when the seeds of *Rhus sp.* and *G. carolinianum* are horizontally cut into a half, radicle in the seeds is pointed into the micropyle (Li *et al*., 1998; Gama-Arachchige *et al*., 2010). However, there is no information of micropyle location on grass pea seeds.

Solute imbibed into seeds (imbibition) is through micropyle and/or seed coat (Manohar and Heydecker, 1964; Edelstein *et al*., 1995; Munz *et al.*, 2017). In seeds of *Cucumis melo* (melon), solute imbibes into the seeds through micropyle, and the seed coat shown by intercellular spaces on the outer layer of seed coat (Edelstein *et al*., 1995). However, when there are no intercellular spaces in the outer layer of the seed coat, the imbibition is only through micropyle as shown in seeds of *G. carolinianum* (Gama-Arachchige *et al*., 2011). However, the information of imbibition pathway on grass pea is still unknown.

The rate of seed imbibition is associated with waterlogging tolerance (Tian *et al*., 2005; Zhang *et al*., 2008); and seed imbibition is related to micropyle (Manohar and Heydecker, 1964). Oxygen dissolved in water or solution that imbibes through micropyle and/or seed coat into embryo is used to produce energy for germination (Chen, 1988; Couee *et al*., 1992; Bewley, 1997; Budko *et al*., 2013). If there is insufficient oxygen, germination may fail (Al-Ani *et al*., 1985) due to low energy production in the embryo (Bewley, 1997; Narsai *et al*., 2011). In addition, waterlogging tolerance in grass pea is related with seed size (Wiraguna *et al*., 2020). However, there is no information on a correlation between micropyle size and waterlogging tolerance.

This study was designed to investigate three hypotheses: (1) micropyle of grass pea seeds is located nearby hilum; (2) water imbibes into grass pea seed through micropyle; and (3) there is a correlation between micropyle size and seed weight of grass pea seeds.

Materials and methods:

The study comprised three experiments was designed to test a role of micropyle during seed imbibition on grass pea seeds. Experiment 1 was to identify a location of micropyle, Experiment 2 was to investigate water penetration during imbibition by covering micropyle with lanolin (Edelstein *et al*., 1995), and Experiment 3 was to test an association between seed weight and micropyle size. These experiments were carried out using electron microscopy in CMCA (Centre for Microscopy, Characterisation and Analysis) for Experiment 1 and in a controlled temperature room (25ºC) for Experiment 2 and 3, University of Western Australia, Perth.

Exp. 1 Seed observation

The Exp. 1 was carried out to identify location of micropyle and other seed tissues. A dry seed of grass pea genotype Ceora were cut longitudinally into half and placed on a cryostage in a JOEL JCM-7000 scanning electron microscopy for observation for ~30 minutes with the cross section facing the sensor of electron microscopy. Identification of micropyle, hilum and radicle were then carried out.

Exp. 2 Water uptake during seed imbibition

The same grass pea genotype as Exp. 1 was tested in Exp. 2 to identify the role of micropyle during seed imbibition and germination. The factor of the Exp. 2 was positions of the lanolin on micropyle (one dot), on opposite side of micropyle (one dot), on micropyle and opposite side of micropyle (two dots) and control (without covering the seed coats). A complete randomised design was applied with three replicates in this experiment. This experiment was conducted in the 25 ºC control temperature room in the dark.

A 10 L solution of 0.5 mM $CaSO_4$ was prepared one day before experiment and placed in the control room temperature (25 ºC). Approximately 1 gram of grass pea seed (genotype Ceora) per treatment per replicate was submerged in a 250 mL flask containing 100 mL of the prepared solution for 24 hours. Seed weight increment was measured as percentage of seed weight increase (Zhang *et al*., 2008).

Two layers of saturated filter papers (Whatman no. 50) were placed on Petri dishes (90 mm diameter). Ten seeds were then put on the saturated filter paper. The seeds were covered with a layer of filter paper and kept in a controlled temperature room at 25 C in the dark for 10 days (Kranner *et al*., 2010; Wiraguna *et al*., 2017; 2020; 2021). Seeds were categorised as germination when the radicles emerged for more than 5 mm in length. There were three replicates for each treatment and observation was carried out daily.

Exp. 3 Association between seed weight and micropyle size of grass pea

Nine grass pea genotypes from different countries of origins were selected to identify a relation between seed weight and micropyle size (Table 1). A one hundred seed weight was recorded after threshing the grass pea pods as described by Wiraguna *et al*. (2017; 2020). Micropyle areas were measured after collecting photographs of seed micropyle under light microscopy with 20X magnificent. The micropyle photograph was then analysed using a computer software (Image J) to measure micropyle areas as describe by Abràmoff *et al*. (2004).

(Table 1 about here)

Data analysis

The data was analysed by analysis of variance (ANOVA) to test the effect of location of lanolin on seed coat in Exp. 2 and by Pearson correlation to test the association between traits in Exp. 3 using R-studio (2022.12.0+353) and GenStat 20th edition (VSN International, UK). Mean differences were indicated by least significant difference (LSD) at $P = 0.05$.

Results:

Exp. 1 Grass pea seed observation

Micropyle showed as a small tunnel connected between inside and outside seeds. The location of micropyle was nearby radicle (Figure 1). Micropyle was located on the end of hilum.

(Figure 1 about here)

Exp. 2 Seed water uptake during imbibition

A one-way ANOVA showed a location of lanolin on the seed coat was significant ($P < 0.05$). The application of lanolin on micropyle significantly reduced percent seed weight by 36.6% relative to control (Figure 2). The seed weight increment between treatments of lanolin application was not significantly different and ranged between 80 and 90%.

(Figure 2 about here)

The treatments of lanolin significantly reduced germination percentage ($P < 0.05$). Control and lanolin placed on the seed coat started germination on the first day of treatment, but germination was delayed for a day for the treatment of lanolin placed on the micropyle (Figure 3). Moreover, germination was significantly lower at <57% for the seeds covered by lanolin on the micropyle relative to the control at 87%.

(Figure 3 about here)

Exp. 3 Association between seed weight and micropyle size of grass pea

There was no significant correlation between seed weight and mycropile size (Figure 4). Therefore, waterlogging tolerance was not associated with micropyle size.

(Figure 4 about here)

Discussion:

Imbibition from the environment to seed embryos has been suggested mainly through micropyle (Manohar and Heydecker, 1964; Munz *et al*., 2017). However, there is limited information on micropyle and the role of micropyle in seed germination. Moreover, a relation between seed weight and micropyle size on grass pea seeds is unknown. In this study, we found the location of grass pea micropyle nearby hilum similar to that shown in seeds of Geranium carolinianum (Gama-Arachchige *et al*., 2011) and Rhus sp. (Li *et al*., 1999). Imbibition rate was lower in seed coat and/or micropyle closure than in control, but there was no difference on seed imbibition rate between treatments (Figure 2). The percent germination was significantly reduced by the closure of micropyle (Figure 3). This finding indicated that the delay of imbibition could result in the failure of germination similar to that shown for field pea (Manohar and Heydecker, 1964; Larson *et al*., 1968; Edelstein *et al*., 1995). The correlation between seed weight and size of micropyle among grass pea genotypes was insignificant (Figure 3).

The location of the micropyle on the seed coat has been found nearby hilum (Figure 1a). This finding is similar to that shown for seeds of *Picea abies* (L.) Karst (Tillman-Sutela and Kuappi, 1994), *Geranium carolinianum* (Gama-Arachchige *et al*., 2010) and soybean (Muramatsu *et al*., 2008).

Imbibition from the environment (outside the seed) to the embryo was suggested mainly through micropyle (Manohar and Heydecker, 1964; Munz *et al*., 2017). The rate of seed imbibition was similar between treatments (lower than the control) (Tillman-Sutela and Kuappi, 1994; Edelstein *et al*., 1995) (Figure 2). The treatment of covering micropyle significantly reduced percent germination (Figure 3). Percent germination was slower for micropyle seeds covered by lanolin than those uncovered by lanolin

(Figure 3). This finding is similar to pea seeds, where seeds failed to germinate or reduced percent germination for micropyle seeds covered by lanolin (Manohar and Heydecker, 1964).

Seed weight and waterlogging tolerance were not associated with micropyle size (Table 1; Figure 4) but both traits were probably related to hilum size (Muramatsu *et al*., 2008). In soybean, for example, small seeds with a large proportion of hilum were more tolerant to waterlogging than large seeds with a large proportion of hilum, presumably, because hilum was used as a reservoir to keep moisture for seeds to germinate and survive during drainage after waterlogging (Tian *et al*., 2005; Muramatsu *et al*., 2008).

Conclusion:

Micropyle and seed coat are pathways of water or solution to enter the seed and reach grass pea seed embryo. The location of micropyle is nearby hilum similar to other seeds such as soybean and *G. carolinianum*. The rate of seed imbibition for 24 hours was lower than control, but the rate of seed imbibition between treatments was not significant. However, percent germination was significantly reduced by covering the micropyle with lanolin. There was no association between micropyle size and seed weight.

Funding:

Author thanks the Indonesia Endowment Fund for Education, the Ministry of Finance of the Republic of Indonesia (LPDP).

Declaration of Competing Interest:

The author declares there is no conflict of interest.

Acknowledgements:

The author is thankful to William Erskine, Timothy Colmer, Imran Malik and Peta Clode for advice on data collection and analysis.

167 Table 1. Name of grass pea genotypes from different country origin

No.	Name of genotypes	Origin	100 seed weight (g)	Response to soil waterlogging
1	8604	Bangladesh	$9.5^{e} \pm 0.1$	Tolerant
2	Ceora	Australia	$14.3^{c} \pm 0.3$	Sensitive
3	CPI 20495	Cyprus	$22.4^{a} \pm 0.1$	Sensitive
4	CPI 9997	Cyprus	$15.6^{b} \pm 0.1$	Sensitive
5	GP. 13	Ethiopia	$11.9^{d} \pm 0.5$	Tolerant
6	GP. 29	Ethiopia	$9.0^{e} \pm 0.1$	Tolerant
7	IFLA 251	Afghanistan	$15.5^{b} \pm 0.2$	Sensitive
8	K 209.12	Pakistan	$6.3^{f} \pm 0.1$	Sensitive
9	Site 41.4	Greece	$8.7^{e} \pm 0.2$	Sensitive

168 Means are followed by standard error (n= 3). Differences in 100 seed weight between genotypes are
169 shown as different letters ($P < 0.001$). Response to soil waterlogging on each genotype referred to
170 Wiraguna *et al*. (2020).
171
172
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Figure 1. Cross section of grass pea seeds indicating micropyle (circled) and hilum (squared) with a scale of 500 µm shown at plate border. The radicle in the seeds shown by an arrow.

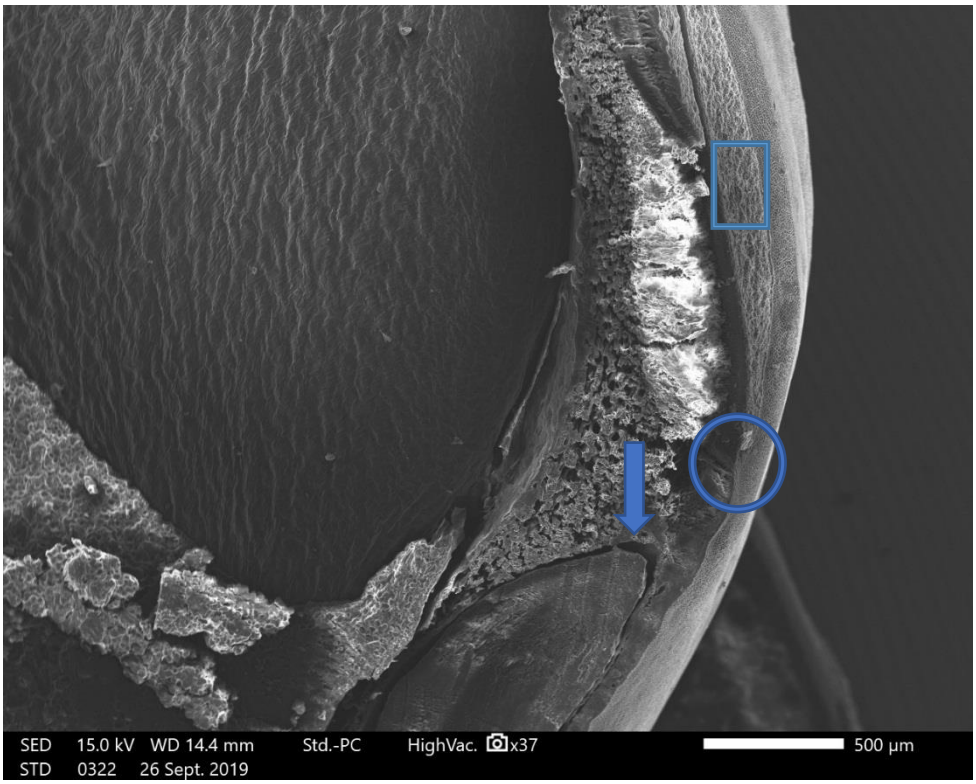


Figure 2. Percent seed weight increment after covering seed coat with lanolin and submergence the seeds for 24 hours in solution of 0.5 mM $CaSO_4$. The lanolin was placed on micropyle (one dot), on opposite site of micropyle (one dot), on micropyle and opposite site of micropyle (two dots) and control (without covering seed coat). Data were means of percent weight increment ± standard from three replicates and 95% confidence interval. The different letters represented a significant difference between treatments at $P < 0.05$ with LSD = 24.6%. The experiment was in a dark controlled temperature room (25 ºC).

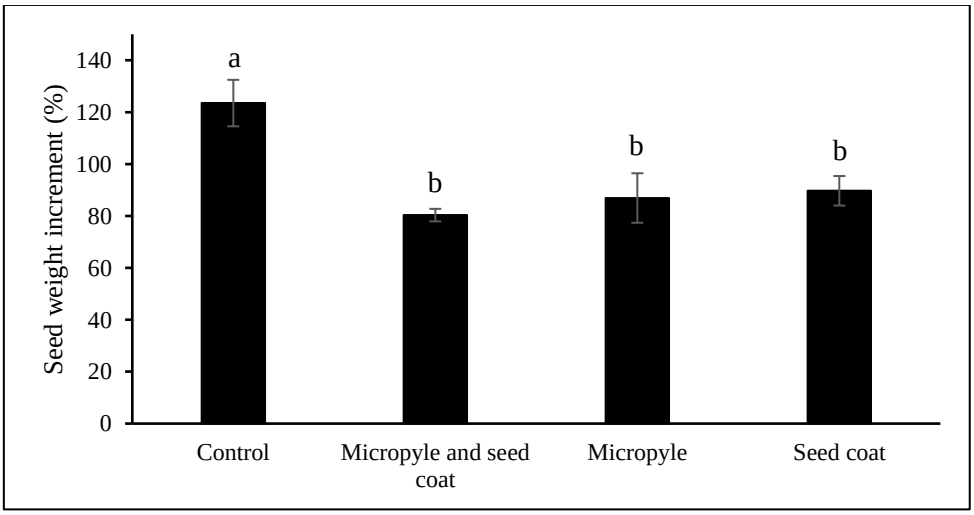


Figure 3. Germination percentage of grass pea seeds with treatments of lanolin placed on micropyle with a square symbol (■), on opposite site of micropyle (seed coat) with a triangle symbol (▲), on micropyle and opposite site of micropyle (seed coat) with a parallelogram symbol (♦) and control with

a circle symbol (●). Data were means of percent germination ± SE from four samples and 95% confidence interval with LSD = 21.8% at day 10 ($P < 0.05$).

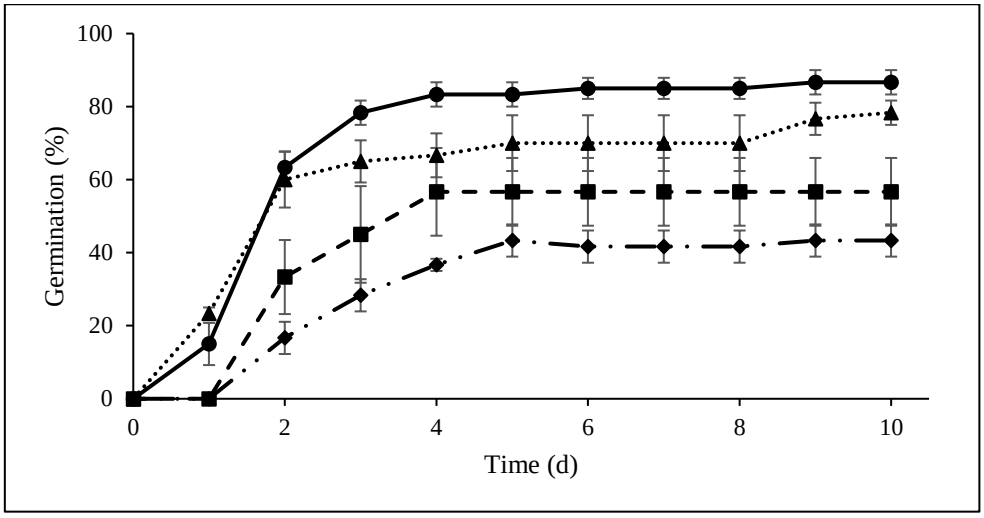
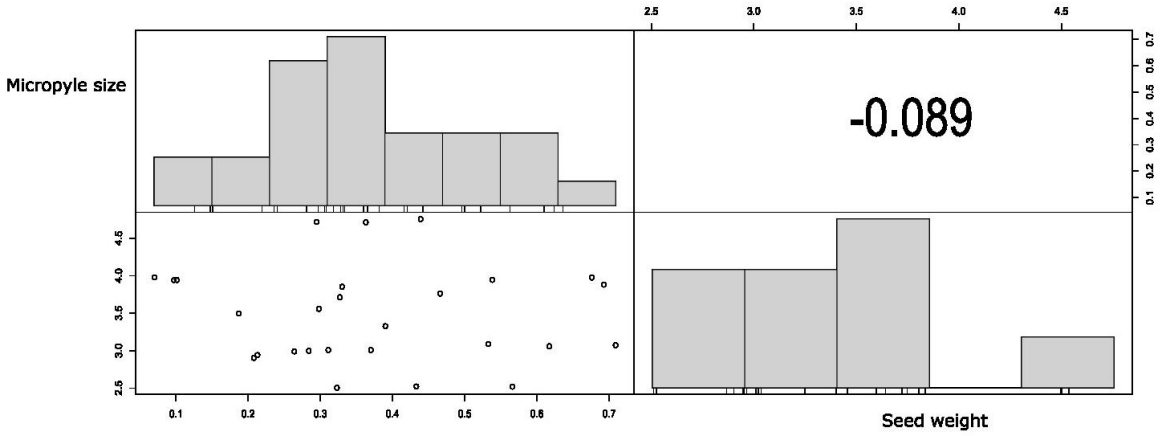


Figure 4. Pearson correlations (r) between seed weight and micropyle area of 9 grass pea genotypes.



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