Waterlogging tolerance of grass pea (Lathyrus sativus L.) at germination related to country of origin

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Abstract
Grass pea (Lathyrus sativus L.) has a Mediterranean origin and was spread to Western Europe, Africa and South Asia. Over time, this grain legume crop has become important in South Asia, where it is often affected by waterlogging at germination. Therefore, varieties with waterlogging tolerance of seeds at germination are needed. This study evaluated waterlogging tolerance in a grass pea diversity panel. First, morpho-agronomic traits of 53 grass pea genotypes from 7 diverse countries (Afghanistan, Australia, Bangladesh, Cyprus, Ethiopia, Greece and Pakistan) were measured in a glasshouse. Seeds of the collection were then sown into waterlogged soil for 6 days and is subsequently drained for 8 days. Finally, representative genotypes from each country of origin of the three survival patterns (described below) were then tested to identify the effect of seed priming on germination and seedling growth in waterlogged soil. Canonical analysis of six traits (seed weight, pod length, pod width, flowering time, time to maturity and seedling survival) showed that genotypes from Bangladesh and Ethiopia were similar. There was a significant variation amongst genotypes in waterlogging tolerance. Genotypes from Bangladesh and Ethiopia showed the highest percent seedling survival (54% and 47%), with an ability to germinate under waterlogging and then maintain growth from the first day of draining to the final sampling (Pattern 1). In contrast, genotypes from other origins either germinated during waterlogging, but did not survive during drainage (Pattern 2) or failed to germinate and had low seedling survival during waterlogging and drainage (Pattern 3). Priming seeds reduced seedling survival in grass pea. Despite Mediterranean origin, specific ecotypes of grass pea with greater waterlogging tolerance under warm wet conditions have been favoured in Bangladesh and Ethiopia where adaptation to extreme precipitation events at germination and seedling survival upon soil drainage is critical for successful crops.

Keywords: Soil flooding; Lathyrus sativus; Genotypic diversity; Origins; Legume crop; Shading; Seed priming

Introduction
Grass pea (Lathyrus sativus L.) originates from the Near East (Mediterranean) where it is grown as a winter crop (Campbell, 1997; Kislev, 1989). The crop was taken to Western Europe, South Asia, e.g. Bangladesh, and Africa, e.g. Ethiopia, where it grows from arid or semi-arid conditions through to waterlogged soil (Campbell, 1997). It is now an important grain legume crop in Bangladesh, Ethiopia and Pakistan (Benková and Zakova, 2001; Campbell et al., 1994; Tadesse and Bekele, 2001; Yigzaw et al., 2001).
Grass pea is valued because of the high protein content (~27%) of its seeds, higher than chickpea (Cicer aritinum L., ~18%) and similar to that of field pea (Pisum sativum L.) and faba bean (Vicia faba L.), serving as a source of protein for the large population in South Asia (Yan et al., 2006). Consumption of grass pea as a primary dietary component for more than 3 months, however, may cause neurolathyrism due to the presence of β-N-oxalyl-L-a,b-diaminopropionic acid (β-ODAP) in the seeds (Campbell, 1997; Yan et al., 2006). Consumption of grass pea has no negative effects when it is a minor component of the diet (<20% of total protein consumption) (Yan et al., 2006).

Grass pea is reputed to be tolerant to abiotic stresses, e.g. drought, flooding and low soil fertility (Benková and Zakova, 2001; Campbell et al., 1994; Tadesse and Bekele, 2001; Yigzaw et al., 2001). Grass pea crops are often exposed to waterlogging at germination in South Asia as farmers broadcast seeds on to soil under near-mature rice crops, a practice called ‘relay cropping’ (Campbell et al., 1994; Gupta and Bhowmick, 2005) – often the soil is saturated from unseasonal rains (Campbell, 1997). In Africa, particularly in Ethiopia, grass pea is grown during the monsoon season, i.e. August or September, in soils with a high clay content, which is prone to water ponding and/or waterlogging (Minta et al., 2014; Tadesse and Bekele, 2001) and it is then subjected to drought during maturity (Campbell, 1997).

Waterlogging causes a decrease and/or failure of germination, as a result of oxygen shortage experienced by seeds (Orchard and So, 1985; Zaman et al., 2018). Exposure to an extended period of waterlogging, i.e. lack of oxygen, causes membrane deterioration and the leakage of cellular contents resulting in germination failure and/or seed death (Johnson et al., 1989; Zaman et al., 2019a). However, seeds of some species can tolerate waterlogging and germinate under water (escape mechanism) or survive and germinate after the water recedes (quiescence mechanism) (Hsu and Tung, 2017; Zaman et al., 2019a). For example, rice (Oryza sativa L.) seeds can germinate and elongate the coleoptile, which can function as a ‘snorkel’ at the water surface, so oxygen is then supplied to the seedling (Hsu and Tung, 2017; Ismail et al., 2009). The second example is field pea seeds with reddish-brown testa colour, which remain quiescent during waterlogging and then germinate upon draining when oxygen is again available (Zaman et al., 2019a).

Seed priming of grain legumes, e.g. lentil and grass pea, can increase seedling establishment and grain yield in relay cropping systems (Ali et al., 2009; Bhowmick et al., 2014). Increased activities of amylase and alcohol dehydrogenase during seed priming are probably the reason for an increase of germination percentage and seedling establishment under relay cropping for primed in comparison with non-primed seeds (Choudhary et al., 2019; Sarkar, 2012). Seed priming leads to faster seedling emergence, earlier flowering and maturity than non-priming in chickpea (Harris et al., 1999). In lentil (Lens culinaris Medik. subsp. culinaris) (Ali et al., 2009; Bhowmick, 2010) and grass pea (Bhowmick et al., 2014), seed priming increased grain yields by 14–17% and 8%, respectively, compared to crops established from non-primed seed under relay cropping. However, there is no information on the interaction of seed priming on survival under waterlogging during germination in grass pea.

Tolerance to waterlogging at germination and emergence has been correlated with morpho-agronomic traits in grain legumes (Sayama et al., 2009; Wiraguna et al., 2017; Zaman et al., 2018). In lentil, for example, early maturity was correlated (r = 0.64) with waterlogging tolerance at germination when seeds were waterlogged for 6 days in a pot soil experiment (Wiraguna et al., 2017). When seeds of the soybean (Glycine max (L.) Merr.) were soaked in distilled water for 48 hours, small seeds showed higher germination and seedling survival than large seeds with a correlation (r) of −0.62 between germination and seed weight, and of −0.57 between seedling survival and seed weight (Sayama et al., 2009). Waterlogging tolerance in small seeds of soybean was associated with high levels of proline-rich proteins in the cell wall of the seed coat which slow water imbibition in small seeds more than in large seeds (Percy et al., 1999; Sayama et al., 2009). However, correlations of morpho-agronomic traits of grass pea with waterlogging tolerance at germination have not been reported.
Given the informal reputation of grass pea, amongst grain legumes, as being waterlogging tolerant, this study was designed to investigate three questions: (1) Does variation in waterlogging tolerance exist in grass pea and does this relate to the genotypic country of origin?; (2) Is waterlogging tolerance correlated with morpho-agronomic traits?; and (3) How does seed priming affect the tolerance of grass pea to waterlogging?

Materials and Methods

The study comprised of three components: Experiment 1 – Morpho-agronomic variation in a diversity panel of grass pea genotypes (Table 1); Experiment 2 – Diversity in waterlogging tolerance at germination in the same panel; and Experiment 3 – Evaluation of the effect of seed priming on waterlogging tolerance on a subsample of the diversity panel.

The experiments were conducted in a glasshouse at the University of Western Australia (UWA), Crawley, Western Australia (31°59'S and 115°49'E). Experiment 1 was conducted from September 2016 to January 2017 when photosynthetically active radiation (PAR) was 897 ± 3 μmol m⁻² s⁻¹ at midday, i.e. 12:00–1:00 pm. Experiments 2 and 3 were conducted between July and August 2018, when the average temperature was 27.7 ± 0.05 °C in the day and 24.2 ± 0.03 °C at night with the range of 20 – 32 °C. Relative humidity (RH) was 44.6 ± 0.18% in the day and 50 ± 0.12% at night with the range of 23 – 66% during Experiments 2 and 3. Shade cloth was used in Experiments 2 and 3 with plants receiving 59 ± 4 μmol m⁻² s⁻¹ of PAR at midday to simulate the low light environment under a dense rice crop canopy as encountered in relay cropping.

Experiment 1 – Morpho-agronomic traits of a diversity panel of 53 genotypes

A diversity panel of 53 genotypes of grass pea from 7 countries with the number of genotypes in parentheses: Afghanistan (9), Australia (3), Bangladesh (3), Cyprus (11), Ethiopia (10), Greece (8) and Pakistan (9) (obtained from the Australian Grains Genebank, Horsham, Victoria) (Table 1) was grown to maturity in a glasshouse in a completely randomised design with three replicates for morpho-agronomic characterisation. The experimental unit was a single pot, diameter 260 mm and height 230 mm. Six seeds were sown in each pot and after 21 days, seedlings were thinned to four plants per pot. Each pot contained ~4.1 kg of potting mix (5:2:3 of pine bark: coco peat: brown river sand). Twenty-eight days after sowing (DAS), 1 g of compound fertiliser (7.3% nitrogen, 11% phosphorus, 28% potassium, 2.8% sulphur, 0.21% iron, 0.1% manganese, 0.08% boron, 0.06% zinc, 0.008% molybdenum) was added to each pot. Forty-nine DAS, this fertiliser was applied twice weekly and the fertiliser application was terminated when 25% of plants formed pods as described by Wiraguna et al. (2017). Watering was terminated when pods were swollen and filled. Plants were harvested when 90% of pods turned a grey colour and leaves turned into yellow at growth stage R8 (Campbell, 1997; Erskine et al., 1990).

Experiment 2 – Screening a diversity panel of 53 genotypes for waterlogging tolerance at germination and seedling establishment

Experiment 2 comprised of two factors: duration of waterlogging and grass pea genotypes in a randomised complete block design (RCBD) replicated 3 times. The first factor – duration of waterlogging – had two levels: 0 (control) and 6 days of waterlogging followed by 8 days of drainage because our preliminary experiment showed that 6 days of waterlogging gave maximum differentiation in waterlogging tolerance amongst grass pea genotypes (data not shown). The second factor comprised of grass pea germplasm with 53 genotypes (Table 1). Seeds were surface sterilised with 1% commercial bleach (active ingredients NaOCl 40 mg L⁻¹) for 1 minute and washed in deionised (DI) water 3 times (~30 seconds in each wash), and fungicide (Tetramethylthiuram disulphide) at the rate of 3 g kg⁻¹ seeds were applied before sowing (Zaman et al., 2018).
Grass pea genotypes in the diversity panel of Experiments 1 and 2, and the subset used in Experiment 3 (Exp. 3), with the country of origin, seed viability (%), germination (%), seedling survival (%) and classified for the pattern of seedling survival when sown into waterlogged soil which was subsequently drained. Pattern of seedling survival was assigned based on percent seedling survival between 4 and 14 DAS and cluster analysis (explained below).

Table 1. Grass pea genotypes in the diversity panel of Experiments 1 and 2, and the subset used in Experiment 3 (Exp. 3), with the country of origin, seed viability (%), germination (%), seedling survival (%) and classified for the pattern of seedling survival when sown into waterlogged soil which was subsequently drained. Pattern of seedling survival was assigned based on percent seedling survival between 4 and 14 DAS and cluster analysis (explained below).

| No. | Name | Country of origin | Exp. 3 | Seed viability (%) | Germination (%) | Seedling survival (%) | Pattern of seedling survival |
|-----|------|------------------|-------|-------------------|----------------|-----------------------|-----------------------------|
| 1   | CPI 24772 | Afghanistan | 97 ± 3.3 | 18 ± 9.7 | 0 ± 0 | 2 |
| 2   | IFLA 235 | Afghanistan | 93 ± 3.8 | 31 ± 9.7 | 2.2 ± 2.2 | 2 |
| 3   | IFLA 239 | Afghanistan | 87 ± 8.8 | 62 ± 10 | 4.8 ± 4.8 | 2 |
| 4   | IFLA 240 | Afghanistan | 80 ± 7.7 | 46 ± 17 | 5.1 ± 2.6 | 2 |
| 5   | IFLA 241 | Afghanistan | 60 ± 7.7 | 52 ± 3.0 | 3.0 ± 3.0 | 2 |
| 6   | IFLA 244 | Afghanistan | 63 ± 6.7 | 6.7 ± 3.3 | 23 ± 14 | 3 |
| 7   | IFLA 246 | Afghanistan | 70 ± 5.8 | 41 ± 20 | 2.6 ± 2.6 | 2 |
| 8   | IFLA 248 | Afghanistan | 80 ± 11.5 | 52 ± 4.8 | 0 ± 0 | 2 |
| 9   | IFLA 251 | Afghanistan | 93 ± 6.7 | 59 ± 2.6 | 0 ± 0 | 2 |
| 10  | Ceora | Australia | 60 ± 5.8 | 49 ± 5.1 | 0 ± 0 | 2 |
| 11  | Chalus | Australia | 100 ± 0 | 36 ± 8.0 | 8.9 ± 8.9 | 2 |
| 12  | IFLA 21 | Australia | 83 ± 6.7 | 51 ± 5.1 | 0 ± 0 | 3 |
| 13  | 8603 | Bangladesh | 70 ± 10 | 40 ± 5.8 | 43 ± 8.8 | 1 |
| 14  | 8604 | Bangladesh | 73 ± 8.8 | 71 ± 8.0 | 47 ± 6.1 | 1 |
| 15  | 8605 | Bangladesh | 47 ± 7.7 | 67 ± 33 | 89 ± 11 | 1 |
| 16  | CPI 10782 | Cyprus | 73 ± 14.5 | 31 ± 19 | 8.9 ± 5.9 | 2 |
| 17  | CPI 16230 | Cyprus | 67 ± 13.3 | 10 ± 6.8 | 0 ± 0 | 3 |
| 18  | CPI 20487 | Cyprus | 83 ± 8.8 | 17 ± 6.3 | 41 ± 6.3 | 1 |
| 19  | CPI 20490 | Cyprus | 93 ± 3.3 | 11 ± 11 | 0 ± 0 | (%) |
| 20  | CPI 20491 | Cyprus | 93 ± 3.3 | 12 ± 5.6 | 2.8 ± 2.8 | 3 |
| 21  | CPI 20492 | Cyprus | 73 ± 8.8 | 15 ± 8.0 | 6.1 ± 3.0 | 3 |
| 22  | CPI 20495 | Cyprus | 90 ± 5.8 | 6.7 ± 3.8 | 13 ± 10.2 | 3 |
| 23  | CPI 9997 | Cyprus | 97 ± 3.3 | 18 ± 5.9 | 8.9 ± 8.9 | 3 |
| 24  | IFLA 320 | Cyprus | 37 ± 8.8 | 0 ± 0 | 17 ± 8.3 | 3 |
| 25  | SEL 471 | Cyprus | 93 ± 3.3 | 26 ± 5.1 | 51 ± 2.6 | 3 |
| 26  | SEL 534 | Cyprus | 87 ± 3.3 | 5.6 ± 5.6 | 11 ± 11 | 3 |
| 27  | GP.13 | Ethiopia | 90 ± 5.8 | 88 ± 4.8 | 83 ± 4.8 | 1 |
| 28  | GP.14 | Ethiopia | 93 ± 3.3 | 76 ± 6.3 | 45 ± 22 | 1 |
| 29  | GP.15 | Ethiopia | 43 ± 3.3 | 76 ± 13 | 15 ± 8.0 | 1 |
| 30  | GP.16 | Ethiopia | 60 ± 10 | 49 ± 13 | 58 ± 17 | 1 |
| 31  | GP.2 | Ethiopia | 93 ± 3.3 | 61 ± 6.1 | 42 ± 11 | 1 |
| 32  | GP.27 | Ethiopia | 90 ± 5.8 | 75 ± 12 | 53 ± 7.3 | 1 |
| 33  | GP.29 | Ethiopia | 80 ± 10.2 | 44 ± 5.1 | 56 ± 24 | 1 |
| 34  | GP.30 | Ethiopia | 73 ± 17.6 | 62 ± 16 | 31 ± 6.3 | 1 |
| 35  | SEL 508 | Ethiopia | 93 ± 6.7 | 52 ± 8.6 | 19 ± 16 | 2 |
| 36  | SEL 526 | Ethiopia | 60 ± 15.3 | 33 ± 9.6 | 36 ± 15 | 1 |
| 37  | CPI 14162 | Greece | 77 ± 8.8 | 0 ± 0 | 39 ± 2.8 | 1 |
| 38  | CPI 14162.1 | Greece | 73 ± 8.8 | 23 ± 11 | 5.1 ± 5.1 | 3 |
| 39  | CPI 14162.3 | Greece | 70 ± 5.8 | 0 ± 0 | 2.8 ± 2.8 | 3 |
| 40  | CPI 31617 | Greece | 80 ± 5.8 | 23 ± 23 | 3.3 ± 3.3 | 3 |
| 41  | IFLA 247 | Greece | 77 ± 8.8 | 83 ± 6.3 | 0 ± 0 | 2 |
| 42  | SEL 38 | Greece | 90 ± 5.8 | 5.1 ± 5.1 | 10 ± 6.8 | 3 |
| 43  | SEL 439 | Greece | 60 ± 6.7 | 11 ± 11 | 7.4 ± 3.7 | 3 |
| 44  | Site 41.4 | Greece | 57 ± 8.8 | 0 ± 0 | 42 ± 30 | 3 |
| 45  | K100.23 | Pakistan | 87 ± 8.8 | 74 ± 4.8 | 0 ± 0 | 2 |
| 46  | K100.24 | Pakistan | 90 ± 5.8 | 100 ± 0 | 0 ± 0 | 2 |
| 47  | K100.31 | Pakistan | 100 ± 0 | 95 ± 4.8 | 0 ± 0 | 2 |
| 48  | K100.32 | Pakistan | 100 ± 0 | 92 ± 8.3 | 8.3 ± 8.3 | 2 |
| 49  | K100.33 | Pakistan | 100 ± 0 | 51 ± 2.2 | 2.2 ± 2.2 | 2 |
| 50  | K100.35 | Pakistan | 100 ± 0 | 59 ± 6.8 | 5.1 ± 5.1 | 2 |
| 51  | K100.36 | Pakistan | 100 ± 0 | 58 ± 12 | 0 ± 0 | 2 |

(Continued)
The diversity panel of 53 genotypes of grass pea was screened for waterlogging tolerance in a pot experiment in free-draining black plastic pots (85 × 85 × 180 mm). Each pot contained ~0.8 kg soil from Mukinbudin, Western Australia with particle size less than 2 mm, pH 8.2 and EC 589 μs m⁻¹ (both in 1:5 soil:water). This substrate has been previously used for waterlogging research on field pea by Zaman et al. (2018; 2019a). A total of 0.76 g compound fertiliser (7.3% nitrogen, 11% phosphorus, 28% potassium, 2.8% sulphur, 0.21% iron, 0.1% manganese, 0.08% boron, 0.06% zinc, 0.008% molybdenum) was applied to the soil in each pot (dissolved and watered when wetting up the soil). Treatment pots were waterlogged for 4 days prior to sowing. Control pots were waterlogged (with DI water) for 3 days and then drained for 1 day to reach field capacity (20.8% water content in soil) prior to sowing.

Fifteen seeds per genotype were placed in each plastic pot, and all pots were placed into a series of plastic tanks, with 18 plastic pots within each 60 L plastic tank. Each pot had been watered, and then 50% of the tanks had DI water added into the tank and the DI water entered the bottom of the pots and rose to above the soil surface in pots in those treatment tanks. The seeds were pressed into the soil, e.g. dibbled, with one side of each seed exposed at the surface, i.e. mimicking relay sowing and allowing sufficient moisture in the drained control pots for seeds to germinate. This dibbled practice also helped to identify seed germination because the radicle could be observed on emergence. Controls (drained pots in tanks without water added to the tanks) were sprayed with DI water for 3 days and then drained for 1 day to reach field capacity (20.8% water content in soil) prior to sowing.

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Low soil oxygen during waterlogging can be inferred from a low redox value (Malik et al., 2015; Patrick et al., 1996; Wiraguna et al., 2017; Zaman et al., 2018). Oxygen was limited for plant growth – e.g. germination failure and reduction of seedling survival – when redox potential was lower than 300 mV (Husson, 2013; Patrick and Mahapatra, 1968). In this experiment, redox values were recorded by inserting platinum electrodes at a depth of 100 mm in 12 pots (6 controls; 6 waterlogged and drained treatments) (Patrick et al., 1996).

**Table 1. (Continued)**

| No. | Name   | Country of origin | Exp. 3 | Seed viability (%) | Germination (%) | Seedling survival (%) | Pattern of seedling survival |
|-----|--------|-------------------|-------|-------------------|---------------|-----------------------|-----------------------------|
| 52  | K100.8 | Pakistan          | X     | 100 ± 0           | 62 ± 2.4      | 4.8 ± 4.8             | 2                           |
| 53  | K209.12| Pakistan          | X     | 100 ± 0           | 81 ± 12       | 0 ± 0                 | 2                           |
| Mean|        |                   |       | 80.1              | 41.9          | 17.4                  |                             |
| F probability (p) | |                   |       | <0.001           | <0.001        | <0.001                |                             |
| LSD |        |                   |       | 21.6              | 28.9          | 25.4                  |                             |

Differences between genotypes were significant for percent seed viability, germination and seedling survival with the least significant differences (LSD) at p = 0.05. Seed viability of each genotype was tested in Petri dishes for 6 days in a temperature-controlled room (25 °C). Germination (%) under waterlogging ± standard error from three replicates were derived after dividing treatment data by a number of germinated seeds of the drained control in Experiment 2. Seedling survival (%) at harvest ± standard error from three replicates was derived after dividing treatment data by the final number of surviving seedlings of the drained control in Experiment 2. Pattern 1 of seedling survival: Genotypes germinated under waterlogging and survived during subsequent drainage. Pattern 2: Genotypes germinated under waterlogging but failed to survive during subsequent drainage. Pattern 3: Genotypes failed to germinate or had low seedling survival under waterlogging and subsequent drainage.

The diversity panel of 53 genotypes of grass pea was screened for waterlogging tolerance in a pot experiment in free-draining black plastic pots (85 × 85 × 180 mm). Each pot contained ~0.8 kg soil from Mukinbudin, Western Australia with particle size less than 2 mm, pH 8.2 and EC 589 μs cm⁻¹ (both in 1:5 soil:water). This substrate has been previously used for waterlogging research on field pea by Zaman et al. (2018; 2019a). A total of 0.76 g compound fertiliser (7.3% nitrogen, 11% phosphorus, 28% potassium, 2.8% sulphur, 0.21% iron, 0.1% manganese, 0.08% boron, 0.06% zinc, 0.008% molybdenum) was applied to the soil in each pot (dissolved and watered when wetting up the soil). Treatment pots were waterlogged for 4 days prior to sowing. Control pots were waterlogged (with DI water) for 3 days and then drained for 1 day to reach field capacity (20.8% water content in soil) prior to sowing.

**Experiment 3 – Evaluating the effect of seed priming on waterlogging response of 26 genotypes selected from the diversity panel**

Experiment 3 comprised of 3 factors: duration of waterlogging (2 levels), seed priming (2 treatments) and grass pea genotypes (26) in an RCBD replicated 3 times. The first factor had two levels of duration of waterlogging: 0 and 6 days followed by 8 days of drainage. The second factor was
seed priming comprising the treatments with or without priming by soaking the seeds in 6 L of DI water overnight (8–10 hours) at 25 °C (Harris et al., 2001; Sarker et al., 2004). The third factor was 26 genotypes (Table 1), selected from Experiment 2 to represent groups with different patterns of seedling survival over time – including both waterlogging tolerant and sensitive genotypes while retaining some diversity for origin. The pot system, soil type and sowing were as described for Experiment 2. The experiment was terminated at 14 DAS.

**Measurements**

In Experiment 1, plants were identified as flowering when more than 50% of the plants in a pot had produced a flower and mature when ~90% of pods formed to grey colour, respectively, (Erskine et al., 1990; Wiraguna et al., 2017). Pod width, pod length, and the number of seeds per pod were recorded according to Campbell (1997) and Polignano et al. (2005). Pods were threshed, and then seeds counted and 100-seed weight (g) was measured as described by Wiraguna et al. (2017).

Seed viability for each genotype was recorded by testing germination of seeds in Petri dishes for 6 days in a temperature-controlled room (25 °C) (Table 1). In Experiments 2 and 3, the numbers of germinated seeds/seedlings were recorded daily with a seed categorised as germinated when a radicle of 5–6 mm was produced (Kranner et al., 2010; Wiraguna et al., 2017). Germination (%) under waterlogging was calculated as the number of germinated seeds during waterlogging at 6 DAS in waterlogged treatment divided by the number of germinated seeds in drained control multiplied by 100. Percent seedling survival was calculated using Equation 1 for Experiment 2 and Equation 2 for Experiment 3 (below), where CT represents drained control and WL represents waterlogged treatment. Soil redox potential was measured daily as described by Wiraguna et al. (2017).

\[
\text{Daily seedling survival} \% = \left( \frac{\text{Number of surviving seedlings in WL}}{\text{Number of surviving seedlings in CT at harvest}} \right) \times 100, \quad (1) \\
\text{Seedling survival at harvest} \% = \left( \frac{\text{Number of surviving seedlings at harvest}}{\text{Number of viable seeds sown}} \right) \times 100. \quad (2)
\]

**Statistical analyses**

The data were analysed by analysis of variance (ANOVA), canonical analysis and restricted maximum likelihood (REML) with an RCBD to test for the effects of treatments, genotypes and interactions using R-studio version 1.0.136 and GenStat edition 18th (VSN International, UK). Significant differences were tested at \( p = 0.05 \).

Hierarchical clustering analysis was carried out in Experiment 2 by R-studio based on percent seedling survival between 4 and 14 DAS as described by Ward (1963). Canonical analysis was computed by contrasting the variation of morpho-agronomic traits between and within countries of origin (Erskine et al., 1989).

**Results**

**Experiment 1 – Morpho-agronomic variation by country of origin**

For all morpho-agronomic traits, except the number of seeds per pod, there were significant differences \( (p < 0.001) \) amongst genotypes and countries of origin (Table 2). Number of seeds per pod was not used further in the analysis. The mean percentage between germination and seedling survival from Bangladesh and Ethiopia was similar, but the mean percentage between germination and seedling survival from Afghanistan and Pakistan was not (Table 2).

Data of seven morpho-agronomic traits were normally distributed (Supplementary Figure S1; Supplementary Table S1). Seedling survival was not significantly correlated with germination (Table 3) and high germination did not always result in high seedling survival (Tables 1 and 2).
Genotypes from Afghanistan, for example, had the highest percent germination but most of these germinated seeds did not survive to the final sampling (Table 2). Therefore, only seedling survival was used further in the analysis. Correlations between seedling survival and the other traits demonstrated that seedling survival was significantly correlated with seed weight and pod length. Correlation between seedling survival and pod length was stronger than between seedling survival and seed weight (Table 3).

Canonical analysis reduced the morpho-agronomic data from six variables (seed weight, pod length, pod width, flowering time, time to maturity and seedling survival) to two orthogonal axes (Figure 1). The first two canonical variates accounted for 94.7% of the total observed variation, with 68.4% and 26.3% of variation explained in canonical variates 1 and 2, respectively. The first canonical variate (CV 1) represented a separation based mainly on seedling survival. The second

Table 2. Multiple comparisons (Fisher’s least significant difference) of 53 grass pea genotypes based on country of origin of seedling survival (%) when seeds are sown into waterlogged soil and with subsequent drainage, germination (%) under waterlogged soil, flowering time (d), maturity time (d), 100-seed weight (g), pod width (cm), pod length (cm) and the number of seeds per pod by one-way ANOVA.

| Country of origin | Seeding survival (%) | Germination (%) | Flowering time (d) | Time to maturity (d) | 100-seed weight (g) | Pod width (cm) | Pod length (cm) | Number of seeds per pod |
|-------------------|---------------------|----------------|-----------------|-------------------|---------------------|--------------|---------------|----------------------|
| Afghanistan (9)   | 4.9b±2.6            | 41.2±4.6       | 43.2±4.1        | 108.9±9.1         | 12.3±0.9            | 1.1±0.1      | 3.8±0.1       | 3.6±0.2              |
| Australia (3)     | 3.0b±3.0            | 30.2±7.2       | 49.2±6.8        | 108.9±6.8         | 14.3±5.9            | 1.5±0.1      | 3.5±0.2       | 3.0±0.6              |
| Bangladesh (3)    | 54.2±6.4            | 59±11          | 46bc±2.8        | 91.2±2.8          | 9.3±1.0             | 0.9±0.0      | 3.0±0.1       | 3.2±0.3              |
| Cyprus (11)       | 10.1±3.8            | 14±2.6         | 46.3±3.3        | 111±3.3           | 15.2±1.1            | 1.2±0.0      | 3.5±0.1       | 3.1±0.1              |
| Ethiopia (10)     | 47.2±6.3            | 62±4.1         | 44±4.7          | 101±4.7           | 7.6±0.2             | 0.9±0.0      | 3.1±0.1       | 3.2±0.1              |
| Greece (8)        | 14.2±5.8            | 18±6.3         | 52±3.9          | 115±3.9           | 18±2.7              | 1.2±0.1      | 3.9±0.1       | 3.2±0.4              |
| Pakistan (9)      | 1.7±0.7             | 73±3.9         | 72±1.1          | 111±1.1           | 8.9±0.3             | 0.9±0.0      | 3.5±0.1       | 3.8±0.2              |
| Mean              | 17.4                | 41.9           | 50.9            | 107.8             | 12.2                | 1.1          | 3.5           | 3.3                  |
| F probability (p) | <0.001              | <0.001         | <0.001          | <0.05             | <0.001              | <0.001       | <0.001         | ns                   |

Values within a column followed by different letters indicate a significant difference with the least significant difference (LSD) at p = 0.05. Differences between countries of origin were significant for all traits, except the number of seeds per pod. Genotype numbers are in parentheses. Data are means followed by the error of three replicates. The traits of flowering time (d), maturity time (d), 100-seed weight (g), pod width (cm), pod length (cm) and the number of seeds per pod were derived from Experiment 1, where plants were grown in a glasshouse without shading (PAR = 897±3 μmol m⁻² s⁻¹ at midday). Seedling survival (%) and germination (%) were derived from Experiment 2, where seeds were sown in a glasshouse under waterlogging for 6 days and drainage for 8 days with 90% shading (PAR = 59±4 μmol m⁻² s⁻¹ at midday). Seedling survival (%) at the final sampling and germination (%) under waterlogging were obtained by dividing treatment data by the final number of surviving seedlings of control in Experiment 2.

Table 3. Pearson correlations (r) between morpho-agronomic traits from 53 grass pea genotypes – flowering time (d), maturity time (d), 100-seed weight (g), pod width (cm), pod length (cm), percent germination under waterlogging and percent seedling survival at the final sampling.

|                      | Time to flowering | Time to maturity | 100-seed weight | Pod width | Pod length | Germination |
|----------------------|------------------|-----------------|----------------|-----------|-----------|-------------|
| Maturity time        | −0.39**          | −0.33*          | −0.81***       | −0.14     | −0.52***  | −0.45***    |
| 100-seed weight      | 0.09             | −0.14           | −0.54***       | −0.16     | −0.58***  | −0.37**     |
| Pod width            | −0.23            | −0.14           | −0.33*         | −0.19     | −0.35**   | −0.01       |
| Pod length           | −0.12            | −0.36**         | −0.52***       | −0.54***  | −0.58***  | −0.37**     |
| Germination          | 0.11             | −0.16           | −0.81***       | −0.33*    | −0.35**   | −0.01       |
| Seedling survival    | −0.21            | −0.23           | −0.33*         | −0.19     | −0.35**   | −0.01       |

The traits of flowering time, maturity time, seed weight, pod width, pod length and the number of seeds per pod were derived from Experiment 1, where plants were grown in a glasshouse without shading (PAR = 897±3 μmol m⁻² s⁻¹ at midday). Seedling survival (%) and germination (%) were derived from Experiment 2, where seeds were sown in a glasshouse under waterlogging for 6 days and drainage for 8 days with 90% shading (PAR = 59±4 μmol m⁻² s⁻¹ at midday). Seedling survival (%) at final sampling and germination (%) under waterlogging were obtained by dividing treatment data by the final number of surviving seedlings of control in Experiment 2.

WL, waterlogging.

*p < 0.05; **p < 0.01; ***p < 0.001.
canonical variate (CV 2) represented the variation mainly based on seed weight (Supplementary Table S2). The canonical analysis emphasised the similarity of genotypes from Bangladesh and Ethiopia, which had high seedling survival after waterlogging, small seeds (Bangladesh 9 ± 1.0 g 100 seeds⁻¹ and Ethiopia 8 ± 0.2 g 100 seeds⁻¹) and pods, and was early to maturity compared to genotypes from other origins (Table 2). By contrast, genotypes from the Mediterranean region (Greece and Cyprus) were also similar to each other and exhibited poor seedling survival after waterlogging (Greece 14 ± 5.8% and Cyprus 10 ± 3.8%), late maturity (Greece 115 ± 3.9 d and Cyprus 111 ± 3.3 d), large seeds (Greece 18 ± 2.7 g per 100 seeds and Cyprus 15 ± 1.1 g per 100 seeds) and long, wide pods (Figure 1 and Table 2).

**Experiment 2 – Screening a diversity panel for waterlogging tolerance at germination and seedling establishment**

Redox measurements showed low soil oxygen during waterlogging, but the oxygen was high following drainage in the controls. In waterlogged pots, the mean redox potential was 226 ± 13 mV (oxygen is limited at redox < 300 mV – Husson, 2013) during waterlogging, but after draining, the redox potential increased to 321 ± 15 mV by 8 days at the end of Experiment 2. In control pots at the start of Experiment 2, the mean soil redox potential was 355 ± 25 mV and this redox potential remained high at 399 ± 28 mV at the end of the experiment.

A one-way ANOVA of seedling survival showed significant differences between genotypes in response to waterlogging at final sampling (p < 0.001) (Supplementary Table S3). Waterlogging had a severe impact on seedling survival where the average survival overall genotypes was only 17% seedlings, relative to control at the final sampling after 8 days of drainage (Table 1). Almost one-quarter of genotypes (13 out of 53) failed to have even a single surviving seedling at final sampling. By contrast, six genotypes namely – GP.13 (Ethiopia), GP.16 (Ethiopia), GP.27

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**Figure 1.** Canonical variate analysis of 53 grass pea genotypes by country of origin using morpho-agronomic traits (Experiment 1 – seed weight, pod length, pod width, flowering time and time to maturity) and percent seedling survival at the final sampling when seed is sown into waterlogged soil and with subsequent drainage (Experiment 2). Cross and plus symbols represent the mean of individual information from the same county of origin and 53 genotypes, respectively. Circles represent confidence limits at p = 0.05.
(Ethiopia), GP.29 (Ethiopia), 8604 (Bangladesh) and 8605 (Bangladesh) – showed a high percent seedling survival after waterlogging and drainage – above 45% (Table 1).

Using the daily surviving seedling data, i.e. daily total number of seedlings during 6 days of waterlogging followed by 8 days of drainage (Supplementary Table S4), a hierarchical cluster analysis was conducted to produce a dendrogram comparing the 53 genotypes (Figure 2a and b). Three major patterns were found with Pattern 1 clustered as waterlogging-tolerant genotypes, and Patterns 2 and 3 were classified as waterlogging-sensitive genotypes (Table 1 and Figure 2a and b). Pattern 1 is composed of genotypes that germinated under waterlogging and continued to survive during drainage. Pattern 2 comprised of genotypes that germinated under waterlogging but did not survive during drainage. Pattern 3 was of genotypes that failed to

![Figure 2. Seedlings (percentage of seedling survival relative to the final number of surviving seedlings of control) for 6 days of waterlogging followed by 8 days of drainage (a) and dendrogram of 53 grass pea genotypes based on similarity for percent seedling survival during waterlogging (6 days) and drainage (8 days) (b) (Experiment 2). Pattern 1 represented for seeds that germinated during waterlogging and survive during drainage, Pattern 2 represented for seeds that germinated during waterlogging but cannot survive during drainage and Pattern 3 represented for seeds that did not germinate or had low seedling survival during waterlogging and during draining. The line in each pattern indicated the fitted value of percent seedling survival. Most seeds started to emerge in control at 3 days after sowing (DAS) shown by black arrows. The dotted lines indicated the difference between seedling patterns.](https://doi.org/10.1017/S0014479720000356)
germinate or had low seedling survival during waterlogging and drainage (Figure 2a). Pattern 1 consisted of 14 genotypes with 1 genotype from Cyprus (CPI 20487), 1 genotype from Greece (CPI 14162), 3 genotypes from Bangladesh and 9 genotypes from Ethiopia (Table 1). All genotypes from Ethiopia, except genotype SEL 508, and from Bangladesh were clustered in Pattern 1 (Table 1 and Figure 2b). Genotypes from Cyprus and Greece showed the broadest diversity since genotypes from these two countries were classified in all three clusters (Table 1).

Experiment 3 – Evaluating the effect of seed priming on seedling survival after waterlogging
Analysis of REML demonstrated that the interaction between seed priming and waterlogging treatment was not significant (Table 4), whereas the main effects for seed priming, genotype and waterlogging and the interaction genotype × waterlogging were all significant. Seed priming reduced seedling survival from 87 to 77% in control and from 19 to 7% in waterlogging treatment (Supplementary Figure S2). The mean seedling survival of primed seeds was 42%, whereas for non-primed seeds, it was 53%. Seed priming had no significant interaction with genotype and waterlogging (Table 4). Interaction between genotype and waterlogging treatment was significant (p < 0.001) confirming the results of Experiment 2 that genotypes differed in waterlogging tolerance (Table 4).

Discussion
Variation in waterlogging tolerance during the seedling stage has been reported within legume species including lupin (Lupinus albus L.) (Davies et al., 2000), faba bean (Solaiman et al., 2007), soybean (Henshaw et al., 2007), lotus (Lotus spp.) (Real et al., 2008) and chickpea (Palta et al., 2010). However, only a few studies have demonstrated variation in waterlogging tolerance at germination in legumes (Malik et al., 2015; Wiraguna et al., 2017; Zaman et al., 2019b). A species comparison using between one and three genotypes, indicated that grass pea is more tolerant to waterlogging at germination than lentil and field pea (Malik et al., 2015). However, there was no information on the variation within grass pea in waterlogging tolerance and whether any such variation might be related to adaptation and selection of grass pea to local environments. In this study, we found that waterlogging tolerance at germination and seedling survival upon soil drainage differed amongst grass pea genotypes; and that genotypes from Bangladesh and Ethiopia were more tolerant than those from other origins.

Genotypes from the Mediterranean region (Greece and Cyprus) were late to maturity and had larger seeds than genotypes from Bangladesh (Table 2). While this study had a relatively low number of genotypes from Bangladesh (3), an earlier study with more than 49 genotypes per country (Rajendran et al., 2018) showed a similar result for morpho-agronomic traits of maturity and seed

### Table 4. Analysis of restricted maximum likelihood (REML) of seedling survival (%) for 26 grass pea genotypes when sown into waterlogged soil and subsequently drained

| Fixed term                              | Wald statistic | ndf | F statistic | ddf | F probability (p) |
|-----------------------------------------|----------------|-----|-------------|-----|------------------|
| Genotype                                | 87.42          | 25  | 3.50        | 192 | <0.001           |
| Waterlogging                            | 597.68         | 1   | 597.68      | 192 | <0.001           |
| Seed priming                            | 32.49          | 1   | 32.49       | 192 | <0.001           |
| Genotype × Waterlogging                 | 139.07         | 25  | 5.56        | 192 | <0.001           |
| Genotype × Seed priming                 | 35.44          | 25  | 1.42        | 192 | 0.099            |
| Seed priming × Waterlogging             | 0.24           | 1   | 0.24        | 192 | 0.628            |
| Genotype × Waterlogging × Seed priming  | 17.69          | 25  | 0.71        | 192 | 0.846            |

A total of 26 genotypes from 7 countries of origin with 3 seedling survival patterns: Pattern 1 (Germination under waterlogging and survival after draining), Pattern 2 (Germination under waterlogging but no survival after draining) and Pattern 3 (Sensitive to waterlogging) were waterlogged for 6 days and drainage for 8 days in a glasshouse under 90% shade (PAR = 59 ± 4 μmol m⁻² s⁻¹ at midday). Seed priming was carried out by soaking seeds in DI water overnight (8–10 h). Seedling survival was derived after dividing treatment data by the number of seeds sown (Experiment 3).

ndf, numerator degrees of freedom; ddf, denominator degrees of freedom.

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weight to this study, where genotypes from Bangladesh mature earlier and have smaller seeds than genotypes from the Mediterranean region. Therefore, despite the low sample size from Bangladesh in this study, morpho-agronomic information of genotypes from Bangladesh can be used to represent this country of origin.

Environmental stress can drive adaptation to local conditions through selection and evolution (Erskine et al., 1989; Hoffmann and Hercus, 2000). Lentil and field pea, for example, originated from the Near East, e.g. northern Syria, with relatively dry and cold conditions during sowing in the month of December (Erskine and El Ashkar, 1993; Gepts, 2004; Silim et al., 1991). In contrast, lentil that was introduced to Bangladesh and field pea that was introduced to Ethiopia have become adapted to waterlogging at germination and to warm conditions through selection because the seeds are sown into waterlogged soil during relay cropping before rice harvest with a mean minimum temperature of ~20 °C in the month of November for lentil, and during high rainfall in the month of July for field pea (Malik et al., 2016; Telaye, 1979; Tsidu, 2012; Wiraguna et al., 2017; Zaman et al., 2019b). Grass pea is suggested to have originated from the same region as lentil and field pea (the Near East) (Campbell, 1997; Kislev, 1989; Larbi et al., 2010). In our experiment, we found significant variation between genotypes from different countries of origin in tolerance to waterlogging, and genotypes from Bangladesh and Ethiopia are more tolerant to waterlogging than those from other origins (Table 2). Genotypes from Bangladesh and Ethiopia were probably selected for waterlogging tolerance at germination by farmers who sow grass pea seeds into the soil with a high soil moisture content (Campbell et al., 1994; Minta et al., 2014; Tadesse and Bekele, 2001). In Bangladesh, grass pea seeds are sown into waterlogged soil under the relay cropping (Campbell et al., 1994) in similar conditions to that shown for lentil (Malik et al., 2016). In Ethiopia, grass pea seeds are sown in a higher rainfall (~160 mm) and warmer temperature (~19 °C) (Minta et al., 2014) than at its origin similar to that described for field pea (Telaye, 1979; Tsidu, 2012).

Amongst correlations, the association between germination under waterlogging and seedling survival after waterlogging for 6 days followed by drainage for 8 days in grass pea was non-significant (Table 3 and Figure S1). Genotypes – mainly from Afghanistan, Australia and Pakistan – germinated under waterlogging but subsequently died during drainage to final sampling. However, genotypes – mainly from Bangladesh and Ethiopia – germinated under waterlogging and survived during the drainage period to final sampling (Table 1 and Figure 2). These different surviving seedling patterns amongst grass pea genotypes illustrate that seedling survival after waterlogging is an important trait in screening for waterlogging tolerance at germination.

A negative correlation between seedling survival after waterlogging and morpho-agronomic traits, e.g. seed weight, was identified (Sayama et al., 2009; Zaman et al., 2018). Greater waterlogging tolerance in small seeds over large seeds may result from small seeds being exposed to higher oxygen partial pressure than large seeds since soil oxygen can decrease substantially within a few millimetres of soil depth (e.g. oxygen profiles in the soil surface of rice; Frenzel et al., 1992). Moreover, the distance between an embryo and a seed coat is closer in small seeds than in large seeds (Borisjuk and Rolletschek, 2009; Rolletschek et al., 2009), so the diffusion path-length is shorter for oxygen to reach the embryo. In this study, we found a similar response, where small grass pea seeds had higher germination and/or seedling survival (Table 2) than large seeds with significant correlations between seed weight and germination \((r = -0.54)\) and between seed weight and seedling survival \((r = -0.33)\) (Table 3). Significant negative correlations between germination and pod length \((r = -0.37)\) and between seedling survival and pod length \((r = -0.35)\) were identified (Table 3). Waterlogging-tolerant genotypes from Bangladesh and Ethiopia have significantly shorter pods than those from other tested origins, and the mean of pod length of genotypes from Bangladesh is 3.0 cm and from Ethiopia is 3.1 cm while the mean of pod length of genotypes from other tested origins is more than 3.5 cm (Table 2). However, there is no clear mechanistic relationship between pod length and waterlogging tolerance at germination amongst countries of origins, apart from the association of these two traits with seed weight (Percy et al., 1999; Sayama et al., 2009).
In addition, seed priming can increase seedling emergence in relay cropping systems (Ali et al., 2009; Bhowmick et al., 2014). However, seed priming significantly reduced seedling survival by an average of 10% in this study. Differing results between relay cropping in a paddy rice field by Bhowmick et al. (2014) and this study were, presumably, due to duration and temperature of seed priming. Grass pea seeds that were previously primed for 6 hours at a minimum of 11 °C night temperature and then sown in relay cropping system (Bhowmick et al., 2014) increased seedling establishment and grain yield. However, when grass pea seeds were primed for 8–10 hours at a temperature of 25 °C, seedling survival was reduced to 42%. The longer duration and warmer temperature of seed priming in this study than in the previous study by Bhowmick et al. (2014) may cause rapid water imbibition (Kader and Jutzi, 2002; Wuebker et al., 2001) and a solute leakage (Zaman et al., 2019b). Therefore, primed seeds showed a lower percent seedling survival than non-primed seeds in this study.

Genotypes of grass pea from Bangladesh and Ethiopia have similar morpho-agronomic traits and are more adapted to waterlogging at germination than genotypes from other tested origins. Even though grass pea originates from the Mediterranean with relatively dry conditions (albeit cool and moist at the typical time of germination), local environment and agricultural practice such as relay cropping have forced grass pea to adapt to wet and warm conditions. Recently unseasonal rains after sowing in November from climate change in Bangladesh have destroyed much of the crop discouraging its cultivation. The identification of waterlogging tolerance gives encouragement to the prospect of improved adaptation. The adaptation of grass pea to wet and warm conditions at germination is encouraging for possible specific selection in other legume crops for improved tolerance to waterlogging at germination.

Conclusion
There was a significant variation amongst grass pea genotypes to waterlogging stress at germination and seedling survival upon soil drainage. Genotypes from Bangladesh and Ethiopia were more adapted to soil waterlogging than those from other origins. The tolerant genotypes were able to germinate under waterlogging and continue growing (during drainage) until the end of the experiment. Genotypes from Bangladesh and Ethiopia had a similarity of morpho-agronomic traits of early maturity, small pods and seeds. Seed priming overnight (8–10 hours) at 25 °C did not increase the tolerance of grass pea seeds to being sown into waterlogged soil.

Supplementary material. To view supplementary material for this article, please visit https://doi.org/10.1017/S0014479720000356.

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Conflict of Interest. The authors declare there is no conflict of interest.

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