

1 Water-logging tolerance in pigeonpea [*Cajanus cajan* (L.) Millsp.]: Genotypic variability and
2 identification of tolerant genotypes

3 Rafat Sultana^{a*}, M. I. Vales^a, K. B. Saxena^a, A. Rathore^a, S. Rao^b, M. G. Mula^a, and R.V.
4 Kumar^a

5 ^aInternational Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru,
6 502324, A.P., India

7 ^bJawaharlal Nehru Krishi Vishwa Vidyalaya (JNKVV), Jabalpur, 482004, M.P., India

8 Corresponding author: Rafat Sultana

9 E-mail*: r.sultana@cgiar.org

10

11 ABSTRACT

12 Pigeonpea is an important legume crop of the semi-arid tropics. In India, pigeonpea is mostly
13 grown in water-logging prone areas resulting in major production losses. It is imperative to
14 identify genotypes which show tolerance at the critical crop growth stages to prevent these
15 losses. A panel of 272 diverse pigeonpea accessions was evaluated for seed level water
16 submergence tolerance for different durations (0 h, 120 h, 144 h, 168 h, and 192 h) under *in-*
17 *vitro* conditions in the laboratory. All genotypes exhibited high (79 to 98.6 %) survival rates
18 for up to 120 h of submergence. After 192 h of submergence, the hybrids as a group,
19 exhibited significantly higher survival rates (79%) than the germplasm (71%), elite breeding
20 lines (68%), and released cultivars (58%). Ninety-six genotypes representing the phenotypic
21 variation observed during the laboratory screening were further evaluated for water-logging
22 tolerance at the early seedling stage using pots, and survival rates were recorded eight days
23 after completion of the stress treatment. Genotypes were further narrowed down from 96 to
24 49 in order to evaluate their performance under natural field conditions. Among the cultivated
25 varieties and hybrids the following were identified as tolerant after three-levels (*In-vitro*, pot

26 and field) of testing: ICPH 2431, ICPH 2740, ICPH 2671, ICPH 4187, MAL 9, ABHAYA,
27 LRG 30, MARUTI, ICPL 20128, ICPL 20237, ICPL 20238, ASHA, and MAL 15. These
28 materials can be used as sources of water-logging tolerance in breeding programs.

29

30 INTRODUCTION

31 Pigeonpea [*Cajanus cajan* (L.) Millsp.] is an important legume crop, mainly grown in the
32 semi-arid tropical (SAT) regions of Asia, Africa, Latin America, and the Caribbean (Saxena,
33 2008). The total global area planted with pigeonpea is 4.5 m ha (FAOSTAT, 2009). India is
34 the number one producer (3.38 m ha) of pigeonpea and imports an additional 400,000 tonnes
35 from Myanmar and Africa to meet domestic needs. Although dozens of pigeonpea varieties
36 have been released, productivity has remained stagnant at around 672 kg ha⁻¹ (FAOSTAT,
37 2009) due to various genetics, management, and biotic and abiotic constraints. Since the area
38 of cultivation is not likely to increase, breeding efforts focusing on breaking the yield barrier
39 through hybrid breeding (Saxena *et al.* 2010) and increasing sustainability of production
40 through incorporating tolerance to major biotic and abiotic stresses are needed to increase
41 production and productivity.

42 Water-logging during the monsoon season (June to September) in India, is caused by erratic
43 and prolonged rains and represents an important production constraint. Since pigeonpea is
44 primarily grown in deep *vertisols* and in the areas with mean annual rainfall of 600-1,500
45 mm, water-logging becomes a serious problem (Chaudhary *et al.* 2011). Water-logging
46 occurs when the water table attains a level at which the soil pores in the root zone of the
47 plants are fully saturated and restrict normal air circulation. Consequently, oxygen level in
48 the soil declines and carbon dioxide concentration increases, which adversely affects the
49 growth and development of plant roots. Drastic reduction in oxygen level and increase in
50 carbon dioxide concentrations are the primary stresses to which the plants are exposed under

51 water-logging conditions (Vartapetian & Jackson, 1997). Inability of aerobic crop species,
52 such as pigeonpea to endure low oxygen conditions at the rhizosphere level, results in
53 substantial yield losses. Roots of most plants are highly susceptible to anaerobic conditions,
54 which support a unique microbial community; and this severely affects the nutrient balance
55 of the soil (Levitt 1980; Laanbroek 1990; Ponnampurna 1972) and plant health. Soon after
56 the onset of short periods of excessive moisture conditions, obligate aerobic bacteria become
57 inactive, and facultative/obligate anaerobic bacteria become active and dominate the micro-
58 flora in the inundated soils (Sachs *et al.* 1980; Jackson 1990). Another adverse effect of
59 water-logging is leaching of important minerals or essential intermediate metabolites from
60 roots into water (Laanbroek 1990; Rathore *et al.* 1997). Water-logging also induces certain
61 changes in physical and chemical properties in the rhizosphere. The gaseous diffusion rates in
62 flooded soils are about 100 times lower than normal (Kennedy *et al.* 1992), and respiration of
63 plant roots, soil micro-flora and fauna leads to rapid exhaustion of soil oxygen, thereby
64 causing anaerobiosis.

65 In India, about 8.5 m ha of arable land is prone to this problem. A recent comparative
66 analysis of pigeonpea growing regions revealed that almost all the states that grow pigeonpea
67 in India are affected by water-logging. It is estimated that around 1.1 mha of the total area
68 under pigeonpea is affected by excess soil moisture, causing an annual loss of 25-30% in
69 production (Chaudhary *et al.* 2011).

70 Considering the important yield losses caused by water-logging in pigeonpea, it is imperative
71 to identify solutions. Although certain soil management options such as the use of raised
72 sloping seed beds, ridge sowing, and transplanting of seedlings, help in reducing losses
73 caused by water-logging (Abebe *et al.* 1992). These options are not economically viable for
74 the resource poor farming community of the SAT. Hence, the use of tolerant genotypes is the
75 most economical and simple way to minimize losses caused by water-logging in pigeonpea.

76 According to Khare *et al.* (2002) the initial establishment of seedlings is the most critical
77 consideration for pigeonpea in water-logging prone areas. Therefore, the objective of this
78 study was to assess the genotypic variability for water-logging tolerance in pigeonpea and to
79 identify genotypes capable of withstanding water-logging stress conditions at sowing and
80 early seedling stages under field prone conditions.

81

82 MATERIALS AND METHODS

83 Critical evaluation of rainfall pattern during the monsoon season (June-September) at
84 Patancheru, Andhra Pradesh, India (latitude 17°32'N; longitude 78°16'E; elevation 545 m)
85 and its overlap with pigeonpea growing stages allowed the most water-logging vulnerable
86 stages as well as the time of occurrence to be identified. Pigeonpea seedlings receive
87 maximum rain during the months of July and August (Fig. 1). Since, the seed (just after
88 sowing), and early seedling stage (15-35 d old seedling) in pigeonpea are very sensitive to
89 water-logging (Fig. 1), the screening methodology was optimized taking into account the
90 crop growth stages that were most severely affected by water-logging.

91

92 **Laboratory screening (seed stage evaluation):** Genotypic variability of 272 pigeonpea
93 genotypes differing in maturity, seed color, seed size and origin were evaluated for water
94 submergence tolerance under laboratory conditions using a simple screening method that
95 allowed evaluation of many genotypes in a short period. The genotypes used in this study
96 consisted of 114 elite breeding lines (ICPLs), 91 germplasm accessions (ICPs), 34 pure line
97 varieties, and 33 Cytoplasmic Male-Sterility (CMS)-based hybrids (ICPHs). All genotypes
98 were obtained from ICRISAT's (International Crops Research Institute for the Semi-Arid
99 Tropics) global gene bank and from the ICRISAT's pigeonpea breeding program (Table 4).

100 Seeds from all the genotypes were collected from the 2009 crop season and stored at 2–4°C

101 until used in the experiment. To avoid the incidence of fungal infection, the seeds were
102 treated with *Thiram* dust (3 g kg⁻¹ seeds) before imposing submergence treatments. The
103 genotypes were classified into different groups based on maturity duration (short, medium or
104 late) and seed coat color (light or dark colored),. The materials included 196 medium to late
105 (160 to 270 d) and 76 early (120 to 155 days to 75% maturity) maturing genotypes. A total of
106 208 genotypes had dark colored (black, purple, dark brown, brown) seeds, while 64 lines had
107 light colored (white, off-white, and cream) seeds. The experiment was conducted under
108 laboratory at ICRISAT, Patancheru, Andhra Pradesh, India during 2009.

109 The genotypes were subjected to water submergence treatments in 200 mL beakers with 10-
110 cm diameter containing 100 mL of water at 23±1⁰C. The submergence treatments were
111 established as a function of the submersion time (S120, S144, S168 and S192 for groups of
112 seeds submerged for 120, 144, 168 and 192 hours, respectively). A baseline (S0 = no
113 submergence treatment) germination test was performed by placing 20 seeds of each
114 genotype between two paper towels in plastic petri-dishes and maintaining humidity as
115 necessary. The durations of S120, S144, and S168 may be comparable to field observations
116 of soil water-logging timing at the study site, especially during rainy years. The S192
117 duration was specifically selected for this experiment in order to check the seed viability
118 under extended (8 days) submergence. Each test sample consisted of 20 seeds and evaluated
119 in three replications. After completing each stress period, the seeds were dried on filter paper
120 for 4 - 5 h to drain excess water and then placed on paper towel in a petri-plate and kept for
121 germination at a constant temperature (25±2⁰C) in a dark room. The seeds were considered
122 germinated when their radicle reached the length of at least 2 mm. The germinated seeds
123 were counted and percent survival was calculated 5-6 days after completing stress treatment.
124 Analysis of variance was performed using SAS software (SAS, 2008) to assess the variation
125 among genotypes, submergence durations and their interactions. The germination data

126 (percent) were arc-sine-transformed (Gomez and Gomez, 1984) to induce linearity in the data
127 set. In addition, further analysis was also performed to compare relative survival rate
128 performance of the four genotypic groups within submergence durations using linear
129 contrasts. The associations of survival rates under the different water-submergence treatments
130 with seed color and maturity duration were assessed using a t-test.

131

132 **Pot screening (early seedling stage evaluation):** 96 out of 272 pigeonpea genotypes
133 representing the four genotypic groups (hybrids, lines, germplasm and varieties) that showed
134 tolerance or moderate tolerance and susceptibility to water submergence at the seed stage
135 during laboratory screening were further evaluated for water-logging tolerance at the seedling
136 stage (15 d old). The evaluation was conducted using 4'' diameter plastic pots perforated at
137 the base at three points with orifices of 5.0 mm diameter. Pots were filled with a mixture of
138 *vertisols*, and farmyard manure (FYM); soil to FYM ratio was 50:1 (V/V). Amount fertilizers
139 (NPK) was calculated on soil weight basis and thoroughly mixed in the soil. Each pot was
140 weighed after filling in order to maintain the same quantity of soil and maintain constant
141 moisture in each pot. For each genotype, five pots were prepared (four pots for imposing
142 stress treatment and one kept as control - no treatment). Filled pots were sown on 24
143 February, 2010, with 5 seeds per pot at 2.0-cm depth using a completely randomized design.
144 All pots were kept in a glass house at an average temperature of $32\pm 2^{\circ}\text{C}$. Before application
145 of water stress treatment, the number of plants in each pot was counted. The stress treatment
146 was imposed by submerging four pots in a tray filled with water in such a way that the pots
147 surface remained at least 2 cm under water for 11 days while the fifth pot was kept at normal
148 moisture as a control. The water level in the trays was kept constant throughout the
149 experiment and maintained for 11 days. Survival rates were recorded eight days after
150 completion of the stress treatment with reference to control. Analysis of variance was

151 performed using SAS software (SAS, 2008) to assess the variation among genotypes for
152 survival rates after imposing stress treatment.

153

154 **Field level evaluation (Screening under natural conditions):** Forty-nine genotypes were
155 further evaluated under natural field conditions to confirm the levels of tolerance observed
156 under laboratory and pot screening. The field trial was conducted at ICRISAT, Patancheru,
157 Andra Pradesh, India on 14 July, 2010 with four replications using a 7 x 7 lattice design in
158 deep *vertisols* on a flatbed rice field with no drainage. Seeds were planted with 50 cm spacing
159 between rows and 25 cm within rows in 4-row plots with 2.5 m long rows. Before planting 46
160 kg nitrogen ha⁻¹ in the form of DAP, was applied as a basal dose. Pre-emergence application
161 of pendimethaline and atrazine mixture (both 0.75 kg ha⁻¹a.i.) was sprayed to keep the crop
162 free from weeds. Soon after sowing, the rains commenced and continued for up to 60 d
163 including 45 rainy days (950 mm rain, and 29 ± 1°C average temperature). Thus, the crop
164 was exposed to continuous natural water stress beginning seven days after sowing with an
165 average water depth of 2.0 ± 1.0 cm and continued for up to 53 d (Fig. 1). The plant survival
166 counts were based on final plant stand at maturity (180 d from sowing). Analysis of variance
167 was performed using SAS software (SAS, 2008) to assess the variation among genotypes for
168 survival rates before harvest.

169

170 RESULTS

171 **Seed stage evaluation**

172 *Effect of submergence durations on seed survival*

173 All genotypes exhibited ≥ 90 % survival rate irrespective of their origin when germinated
174 under normal moisture conditions (S0, control = no submergence) (Fig. 2). The analysis of
175 variance showed highly significant differences (p <0.01) among genotypes for seed survival

176 rates for all submergence durations (Table 1). There were also significant survival rate
177 differences among the various submergence durations (S120, S144, S168, and S192). The
178 interaction between genotypes and submergence durations was also significant; therefore
179 further analysis was carried out to understand genotypic performance at each submergence
180 duration. This analysis revealed that the variation among genotypes for survival rate was
181 highly significant at all the submergence durations (Table 2). To explore further, the four
182 distinct genotypic groups (hybrids, germplasm, breeding lines, and varieties) were compared
183 using linear contrasts. Significant differences between groups for survival rates were recorded
184 for the submergence durations. However, as individual group; germplasm and hybrid, as well
185 as varieties and lines were found statistically similar at S144, whereas at S192 the lines and
186 germplasm performed in a similar way (Table 3). The analysis further revealed that after 120
187 h treatment the genotypes, irrespective of their origin, had high (> 80%) mean survival rates.
188 Even after 168 h of submergence the mean survival rate was 73%, which suggested that most
189 of the genotypes had potential to tolerate severe submergence stress. A sharp decline in seed
190 viability was observed at the 192 h submergence period (Fig. 2). After 192 h of submergence
191 the hybrids exhibited highest survival rate (>79%) followed by germplasm accessions (71%),
192 advanced breeding lines (68%), and released varieties (> 58%) (Fig. 3).

193

194 ***Relationship of maturity, seed color, and seed weight with survival rate***

195 Medium to late maturing genotypes, irrespective of their origin, had significantly ($p < 0.01$)
196 higher mean survival rate (69.9%) than that of short maturity types (41.7%) (Fig. 4). Further
197 group-wise analysis revealed that in general the medium to late maturing inbred lines had
198 higher survival rates (78.3%) than short (45.3%) maturing types. Similar results were
199 recorded among germplasm and varieties. However, hybrids exhibited consistently high
200 survival rate irrespective of their maturity groups. It was also observed that the mean survival

201 rate was significantly higher in the genotypes with dark colored seed coats (64.5%) in
202 comparison with light colored seed coats (54.4%). In addition to maturity and seed coat color,
203 the seed size was found to be positively associated ($p < 0.05$) with survival rate of the
204 genotypes at all the levels of submergence treatment, S120 ($r = 0.234^*$), S144 ($r = 0.196^*$),
205 S168 ($r = 0.163^*$) and S192 ($r = 0.152^*$).

206 Based on the results of laboratory survival rates, the genotypes were classified into four
207 groups (Table 4); tolerant (>75%), moderately tolerant (50-74%), moderately susceptible (25-
208 49%) and susceptible (<25%). Survival rate at the S192 duration, varied from 20 to 100, 2 to
209 100, 2 to 100 and 0 to 93.3 in hybrids, germplasm, elite inbred lines, and varieties,
210 respectively.

211

212 **Evaluation at early seedling stage**

213 Ninety-six pigeonpea genotypes including tolerant (46), moderately tolerant (10) and
214 susceptible (40) were further evaluated at the seedling stage for water-logging tolerance.
215 Analysis of variance revealed highly significant differences ($p < 0.01$) among the genotypes
216 for seedling survival which ranged from 0 to 95 % (Table 4 and Fig. 5). Most of the
217 genotypes (54) tested for survival rate at early seedling stage in pots were found to be
218 sensitive to water-logging and only a few genotypes exhibited high (up to 100%)
219 germination. The most tolerant genotypes had dark seed color, medium to late maturing type
220 and had 100 seed weight > 10 g.

221

222 **Field evaluation**

223 The forty-nine genotypes screened under natural field conditions showed significant
224 variability for survival rate. A subset of genotypes which showed a high level of water-
225 logging tolerance in all the three levels of screenings (Laboratory, Pot, and field screening)

226 during 2009 and 2010 years were: early - ICPH 2431, medium -ICPH 2740, ICPH 2671,
227 ICPH 4187, Asha, Abhaya, LRG 30, Maruti, ICPL 20117, ICPL 20125, ICPL 20128, ICPL
228 20237, ICPL 20238, ICPL 99050, and late maturity- ICPL 20092, MAL 9, and MAL 15
229 (Table 4). All the tolerant genotypes had dark seed color with 100 seed weight > 10 g.

230

231 DISCUSSION

232 The diverse rainfall patterns in India render the country highly vulnerable to floods as more
233 than 90% of pigeonpea is grown under rainfed condition (Saxena 2008). Among the abiotic
234 stresses that affect pigeonpea, water-logging during seed germination, seedling establishment,
235 and early vegetative growth stage result in poor plant stands (Duke & Kakefuda, 1981) which
236 leads to significant yield losses and instability in production (Reddy & Virmani, 1981).
237 Water-logging during germination and emergence generally results in poor plant stands.
238 According to Powell & Mathews (1978) injury to the seeds is caused by excessive water
239 accumulation due to rapid water absorption.

240 In the present *in-vitro*, pot, and in field screening of pigeonpea, genotypes for water-logging
241 tolerance observed the significant differences for survival rates, indicating the presence of
242 large genotypic variability (Table 4). The genotypic differences for water-logging tolerance at
243 seedling level in pigeonpea were also studied by Dubey & Asthana 1987; Tekele & McDavid
244 1995; Chauhan *et al.* 1997; Perera *et al.* 2001; Sarode *et al.* 2007; and Krishnamurthy *et al.*
245 2011. In the present study 68% and 44% of the pigeonpea genotypes evaluated at seed and
246 early seedling stages respectively were found tolerant, the survival rates reduced drastically,
247 with increased duration of soaking in laboratory tests. Some of the susceptible materials
248 started deteriorating after 120 h of soaking (Fig. 2). The reductions in survival rate under
249 prolonged submergence were attributed to anoxia/hypoxia (Orchard & Jessop 1984). Oxygen
250 deprivation, either complete (anoxia) or partial (hypoxia) is detrimental to most species of

251 higher plants, inevitably raising the question of whether there are some fundamental
252 physiological differences among plants in their cellular responses to the imposed
253 anaerobiosis. It is often assumed that most cultivated species avoid, rather than tolerate the
254 oxygen shortages (Armstrong *et al.* 1994). Respiration and electron transport under anoxic
255 conditions are inhibited and adenosine tri-phosphate (ATP) formation is decreased (Johnson
256 *et al.* 1989; Tsai *et al.* 1997) which results in decreased seed viability and poor germination.
257 In the present study, the survival rate was found to be related with the origin of genotypes
258 (Fig. 3). Among the four contrasting genotypic groups, the hybrids exhibited greater survival
259 rates compared to germplasm accessions, elite inbred lines, or varieties. In most of the
260 genotypic groups the reduction in survival rates was more or less similar after each
261 submergence period, but the maximum reduction in survival was recorded in the pure line
262 varieties (Fig. 3). This may be due to differences in the imbibition rates and the amounts of
263 reserved materials present in the seeds. Significant varietal differences in response to
264 flooding tolerance have been reported in maize (*Zea mays* L.) and it was found that hybrids
265 performed better than inbred lines under excess soil moisture conditions (Sultana *et al.* 2009).
266 This was attributed to the fact that hybrid seeds may have experienced less oxygen
267 deprivation during submergence as compared to pure lines. It may also be related to relatively
268 high initial vigor or more food reserves in the hybrids (data not published). In pigeonpea
269 hybrids, such variability might also be related to the ability of hybrids to utilize the stored
270 assimilates through anaerobic metabolism during germination and early seedling growth.
271 After evaluation for water-logging tolerance in the laboratory, pot and field levels, medium to
272 late maturing genotypes had higher survival rate compared to short duration types that may
273 be related to their seed size (Fig. 4). Short duration pigeonpea were more sensitive to short
274 term water-logging because they have less time to recover from this stress in comparison to
275 long duration types Matsunaga *et al.* (1994). In general the mean survival rate was

276 significantly higher among the genotypes which had dark seed coat color than that of cream
277 to white seed color after 192 h of water-stress treatment (Fig. 4). Thus, it is concluded that the
278 dark-seeded genotypes tolerate waterlogged situation better than light seeded materials.
279 Similar results were reported by Khare *et al.* (2002) in pigeonpea and they attributed it to
280 slow rate of water uptake due to high levels of phenolic and tannin compounds in their seed
281 coat.

282 Besides origin, maturity, or seed coat color, the seed size of genotypes played a significant
283 role in survival after different water-submergence treatments. However, in general a decrease
284 in survival rate was recorded after S192 treatment in small seeded elite inbred lines. The
285 marked differences in rates of survival may be related to different rates of imbibition in
286 different seed sizes. The small seeds have large contact surface area which may facilitate fast
287 water movement through micropyle compared to large-seeded genotypes (de Jabrun *et al.*
288 1980).

289 The water-logging tolerant genotypes identified through natural field screening included
290 hybrids (ICPH 2431, ICPH 2671, ICPH 2740, and ICPH 4187), varieties (Asha, LRG 30,
291 Maruti, MAL 9, MAL 15 and Abhaya) and advanced breeding lines (ICPL 20092, ICPL
292 20117, ICPL 20125, ICPL 20128, ICPL 20237, ICPL 20238, and ICPL 99050). These
293 genotypes are high yielding as well as resistant to major diseases. Therefore, we propose to
294 promote these genotypes (after on-farm validation) in the area prone to water-logging and
295 envisage that farmers will be able to harvest good yields under temporary water-logged
296 conditions. This will eventually lead to reduction in overall losses caused by water-logging in
297 pigeonpea. Highly tolerant and susceptible genotypes can also be used as parental lines to
298 generate mapping populations in order to study the genetics of traits linked to water-logging
299 tolerance in pigeonpea, subsequently facilitating the introgression of water-logging tolerance

300 into different pigeonpea backgrounds by using a combination of conventional and molecular
301 breeding approaches.

302

303 ACKNOWLEDGMENTS

304 The authors would like to thank Dr C. L. L. Gowda, Director, Grain Legumes Research
305 Program, ICRISAT for providing valuable inputs and suggestions to strengthen this
306 manuscript. The authors are highly thankful to the National Food Security Mission, New
307 Delhi, India for providing funds to carry out this study.

308

309

310

311

312

313

314

315

316

317

318

319

320

321

322

323

324

325 REFERENCES

- 326 Abebe, M., Mamo, T., Duffera, M. & Kidam, S. (1992). Durum wheat response to improved
327 drainage of Vertisol in the central highlands of Ethiopia. In *Seventh Regional Wheat*
328 *Workshop for Eastern* (Eds, D.G.Tanner & W. Mwangi), pp. 407-414. Central and
329 Southern Africa, Nakuru, Kenya.
- 330 Armstrong, W., Brandle, R. & Jackson, M.B. (1994). Mechanism of flood tolerance in
331 plants. *Acta Botanica Neerlandica* **43**, 307-358.
- 332 Chaudhary A.K., Sultana, R., Aditya, P., Nadarajan, N. & Jha, U.C. (2011). Breeding
333 for abiotic stresses in pigeonpea, *Journal of Food Legumes* **24**, 165-174.
- 334 Chauhan, Y.S., Silim, S.N., Kumar Rao, J.V.D.K. & Johansen, C. (1997). A pot technique
335 to screen pigeonpea cultivars for resistance to water-logging. *Journal of Agronomy*
336 *and Crop Science* **178**, 179-183.
- 337 de Jabrun, P.L.M., Byth, D.E. & Wallis, E.S. (1980). Imbibition by and effects of
338 temperature on germination of mature seed of pigeonpea. In *International*
339 *Workshop in Pigeonpea* (Eds. Y.L. Nene), pp. **2**, 181-188. *Proceedings of the*
340 *International Workshop on Pigeonpea, ICRISAT, Patancheru, India.*
- 341 Dubey, S.D. & Asthana, A.N. (1987). Selection of plant type resistance to waterlogging
342 in pigeonpea. In *Food Legume Improvement for Asian Farming Systems.*
343 *Proceedings of an International Workshop held in Khon Kaen, Thailand, 1-5*
344 *September 1986* (Eds E. S. Wallis & D. E. Byth), p. 311. Canberra, Australia:
345 ACIAR.
- 346 Duke, S.H. & Kakefuda, G. (1981). Role of testa in preventing cellular rupture during
347 imbibitions of legume seeds. *Plant Physiology* **67**, 449-456.
- 348 FAOSTAT.2009. <http://faostat.fao.org/site/567/DesktopDefault.aspx?PageID=567#ancor>
349 (verified 26th September. 2010).

- 350 Gomez, K.A. & Gomez, A.A. (1984). Statistical Procedures for Agricultural Research.
351 John Wiley and Sons (1984), 2nd edition, paperback, pp. 680. New York,
352 Chichester, USA.
- 353 Jackson, M.B. (1990). Hormones and developmental changes in plants subjected to
354 submergence and soil water-logging. *Aquatic Botany*, **38**, 49-72.
- 355 Johnson, J., Cobb, B.G. & Drew, M.C. (1989). Hypoxic induction of anoxia tolerance in
356 root tips of *Zea mays* L. *Plant Physiology* **91**, 837-841.
- 357 Kennedy, R.A., Rumpho, M.E. & Fox, T. C. (1992). Anaerobic metabolism in plants.
358 *Plant Physiology* **100**, 1-6.
- 359 Khare, D., Rao, S. Lakhani, J.P. & Satpute, R.G. 2002. Tolerance for flooding during
360 germination in pigeonpea. *Seed Research* **30**, 82-87.
- 361 Krishnamurthy, L. Upadhyaya, H. D., Saxena, K. B. & Vadez, V. (2011). Variation for
362 temporary waterlogging response within the mini core pigeonpea germplasm.
363 *Journal of Agricultural Science*, Cambridge 1-8.
- 364 Laanbroek, H. J. (1990). Bacterial cycling of minerals that affect plant growth in
365 waterlogged soils: a review. *Aquatic Botany* **38**, 109-125.
- 366 Levitt, J. (1980). Excess water or flooding stress. In *Responses of plants to environmental*
367 *stresses*. Academic Press, New. York **2**, 213-228.
- 368 Matsunaga, R., Osamu, I., Satoshi, T., Rao, T. P. & Johansen, C. (1994). Response of
369 short-duration pigeonpea to nitrogen application after short-term water-logging on
370 a vertisol. *Field Crops Research* **38**, 167-174.
- 371 Orchard, P.W. & Jessop, R.S. (1984). The response of sorghum and sunflower to short
372 term water-logging. I. Effects of stage of development and duration of water-
373 logging on growth and yield. *Plant Soil* **81**, 119-132.

- 374 Perera, A.M., Pooni, H.S. & Saxena, K.B. (2001). Components of genetic variation in
375 short-duration pigeonpea crosses under waterlogged conditions. *Journal of*
376 *Genetics and Breeding* **55**, 21-38.
- 377 Ponnampuruma, F. N. (1972). The chemistry of submerged soil. *Advances in Agronomy*
378 **24**, 29-95.
- 379 Powel, A.A. & Mathews, S. (1978). The deterioration of pea embryo during imbibitions.
380 *Journal of Experimental Botany* **29**, 1215-1229.
- 381 Rathore, T.R., Warsi, M.Z.K., Zaidi, P.H. & Singh, N.N. (1997). Water-logging problem
382 for maize production in Asian region. *TAMNET News Letter* **4**, 13-14.
- 383 Reddy, S.J. & Virmani, S.M. (1981). Pigeonpea and its climatic environment. In Y.L.
384 Nene (ed.) *Proceedings of the International Workshop on Pigeonpea. Patancheru,*
385 *India.* **1**, 15-19.
- 386 Sachs, M.M., Freeling, M. & Okimoto, R. (1980). The anaerobic proteins in maize. *Cell*
387 **20**, 761-767.
- 388 Sarode, S.B., Singh, M.N. & Singh, U.P. (2007). Genetics of water-logging tolerance in
389 pigeonpea [*Cajanus cajan* (L.) Millsp]. *Indian Journal of Genetics and Plant*
390 *Breeding* **67**, 264-265.
- 391 SAS Institute Inc (2008). SAS/STAT® 9.2 User's Guide, Cary, NC: SAS Institute Inc.
392 (2008)
- 393 Saxena, K.B. (2008). Genetic improvement in pigeonpea- a review. *Tropical Plant Biology*
394 **1**, 159-178.
- 395 Saxena, K.B. Sultana, R., Mallikarjuna, N., Saxena, R. K., Kumar, R. V., Sawargaonkar,
396 S. L. & Varshney, R. K. (2010). Male-sterility systems in pigeonpea and their role
397 in enhancing yield. *Plant Breeding* **129**, 125—134.

- 398 Sultana, R., Singh, P.P., Singh, R.P., Singh, D. K., Jat, M.L., Dass, S., Zaidi, P.H. &
399 Singh. I. (2009). Studies on gene effects of traits associated with excessive soil
400 moisture tolerance in tropical maize (*Zea mays* L.). *Proceedings of 4th World*
401 *Congress on Conservation Agriculture” New Delhi, India.* P.81
- 402 Takele, A. & McDavid, C.R. (1995). The response of pigeonpea cultivars to short
403 durations of water-logging. *African Crop Science Journal* **3**, 51-58.
- 404 Tsai, C.F., Chu, T.M. & Wang, C.Y. (1997). Effect of water-logging on growth and
405 development of sorghum plant: Responses of seed germination. *Chinese Agronomy*
406 *Journal* **7**, 203-212.
- 407 Vartapetian, B. B. & Jackson, M. B. (1997). Plant adaptations to anaerobic stress. *Annals*
408 *of Botany* **79**, 3-20.
- 409
410
411
412
413
414
415
416
417
418
419
420
421
422

423 Figures legend:

424 Fig. 1: The average rainfall distribution at Patancheru (17⁰N, 78.47E, 545 m), India, from the
425 last 10 years and during the 2010 pigeonpea growing season. The rectangles indicate the
426 duration of the crop growing stages potentially affected by water-logging.

427

428 Fig. 2: During seed stage evaluation, survival rate of 272 pigeonpea genotypes after, 120,
429 144, 168 and 192 hours of water submergence, where bin 1 = 0-10%, 2 = 10-20%, 3 = 20-30
430 10 = 100 %, survival rate.

431

432 Fig. 3: Survival rates (with 95% confidence interval) of the different groups of genotype
433 submerged for 120 (S120), 144 (S144), 168 (S168), and 192 (S192) hours under water,
434 during seed stage screening, (G= germplasm; H=hybrids; L=lines, and V= varieties)

435

436 Fig. 4: Survival rate of pigeonpea genotypes (grouped based on maturity duration and seed
437 coat color) after 192 h of water submergence treatment under laboratory screening; where
438 least significant differences (LSD, 0.05) for maturity duration and seed coat color was 5.8
439 and 6.7 respectively.

440

441 Fig. 5: After seedling stage evaluation (pot screening), survival rates of 96 pigeonpea
442 genotypes after completion of submergence treatment, where bin 1 = 0-10%, 2 = 10-20%, 3 =
443 20-30 10 = 100 %, survival rate.

444

445

446

447

448 Table 1: Analysis of variance of 272 pigeonpea genotypes for survival rate under four water
449 submergence durations at seed stage.

Source	Degree of Freedom	Mean Sum of Square
Genotypes (G)	271	1.17**
Submergence duration (S)	3	19.54**
G x S	813	0.09**
Error	1088	0.04
Corrected total	2181	

450 ** significant at $p < 0.01$ probability

451 Table 2: Comparison of pigeonpea genotypes for survival rate within each water
452 submergence treatments (S) at seed stage screening.

Submergence durations	Source	Mean Sum of Square
120 h (S120)	Genotypes(G)	0.310**
144 h (S144)	Genotypes (G)	0.342**
168 h (S168)	Genotypes (G)	0.385**
192 h (S192)	Genotypes(G)	0.419**

453 ** Significant at $p < 0.01$ probability

454 Table 3: Comparison of pigeonpea genotypes (hybrids, lines, varieties and germplasm) for
455 survival rate using linear contrasts at seed stage screening.

Contrast	Degree of Freedom	Mean Sum of Square			
		120 h	144 h	168 h	192 h
Hybrids vs varieties	1	0.84**	0.75**	2.78**	3.01**
Hybrids vs lines	1	2.22**	0.81**	2.14**	1.34**
Hybrids vs germplasm	1	0.26**	0.01 ^{NS}	0.55**	0.78**
Varieties vs lines	1	0.15*	0.03 ^{NS}	0.34**	0.96**
Varieties vs germplasm	1	0.36**	0.89**	1.65**	1.47**
Lines vs germplasm	1	1.86**	1.20**	0.99**	0.13 ^{NS}

456 *, ** significant at $p < 0.05$ and $p < 0.01$ probability, respectively; NS= non-significant

457

458

459

460

461 Table 4: Pigeonpea genotypes representing tolerant (75-100%), moderately tolerant

462 (50-74%), moderately susceptible (25-49%) and susceptible (<25%) on the basis of survival

463 rate after the 192 h water submergence treatment at seed stage screening.

464

Survival rate (%)	Genotypic groups	Pigeonpea genotypes screened for water-logging tolerance					
Tolerant (100-75)	Elite inbred lines	ICPA 2039	ICPL 99051	ICPL 20100	ICPL 20118	ICPL 20129	
		ICPL 150	ICPL 99054	ICPL 20103	ICPL 20119	ICPL 20130	
		ICPL 332	ICPL 99055	ICPL 20107	ICPL 20120	ICPL 20131	
		ICPL 83057	ICPL 99061	ICPL 20108	ICPL 20121	ICPL 20132	
		ICPL 86005	ICPL 20058	ICPL 20109	ICPL 20122	ICPL 20133	
		ICPL 87051	ICPL 20092	ICPL 20110	ICPL 20123	ICPL 20236	
		ICPL 9048	ICPL 20093	ICPL 20112	ICPL 20124	ICPL 20237	
		ICPL 92043	ICPL 20094	ICPL 20113	ICPL 20125	ICPL 20238	
		ICPL 93101	ICPL 20095	ICPL 20114	ICPL 20126	ICPL 20241	
		ICPL 99046	ICPL 20096	ICPL 20116	ICPL 20127	ICPL 20242	
	ICPL 99050	ICPL 20099	ICPL 20117	ICPL 20128	ICPL 20243		
	Hybrids and Varieties	<i>Asha</i>	ICPH 2740	ICPH 3629	ICPH 4104	MAL 11	
		BDN 1	ICPH 3341	ICPH 3740	ICPH 4187	MAL 15	
		BRG1-(w)1	ICPH 3362	ICPH 3766	ICPH 4301	MAL 9	
		ICPH 2431	ICPH 3371	ICPH 3964	ICPH 4322	SIPS 15	
		ICPH 2671	ICPH 3461	ICPH 3992	JBP 110-B	SIPS 18	
		ICPH 2673	ICPH 3481	ICPH 4031	LRG 30	SIPS 9	
	Germplasm	ICP 10948	ICP 12176	ICP 13384	ICP 14318	ICP 7597	
		ICP 11059	ICP 12739	ICP 13389	ICP 1571	ICP 7815	
		ICP 11130	ICP 12740	ICP 13391	ICP 2376	ICP 7977	
		ICP 11378	ICP 1275	ICP 13392	ICP 4924	ICP 8465	
		ICP 11811	ICP 12750	ICP 13395	ICP 5028	ICP 8466	
		ICP 11813	ICP 12751	ICP 14085	ICP 5429	ICP 8927	
		ICP 12023	ICP 12839	ICP 14092	ICP 7086	ICP 8929	
		ICP 12024	ICP 13361	ICP 14146	ICP 7193		
	ICP 12043	ICP 13379	ICP 14282	ICP 7201			
	Moderately tolerant (50-74)	Elite inbred lines	ICPB 2039	ICPL 20101	ICPL 20106	ICPL 20244	ICPL 96061
			ICPL 161	ICPL 20102	ICPL 20135	ICPL 87154	
ICPL 20097			ICPL 20104	ICPL 20219	ICPL 90030		
ICPL 20098			ICPL 20105	ICPL 20229	ICPL 92059		
Hybrids and		BRG 2	ICPH 2741	ICPH 4329	SGBS 6	UPAS 120	
		ICPH 2363	ICPH 3313	JBP 36B	SIPS 10		

	Varieties	ICPH 2364	ICPH 4183	<i>Maruti</i>	SIPS 17		
		ICPH 2438	ICPH 4275	SGBS 4	SIPS 5		
	Germplasm	ICP 10960	ICP 11296	ICP 14304	ICP 1575	ICP 8094	
		ICP 10987	ICP 1141	ICP 14410	ICP 1941	ICP 8920	
		ICP 11128	ICP 11440	ICP 14712	ICP 4928		
		ICP 11133	ICP 12057	ICP 14882	ICP 5529		
ICP 11150		ICP 13342	ICP 15200	ICP 7741			
Moderately susceptible (25-49)	Elite inbred lines	ICPL 20200	ICPL 20222	ICPL 84060	ICPL 90034	ICPL 990091	
		ICPL 20218	ICPL 84031	ICPL 87091	ICPL 95040		
	Hybrids and Varieties	ICPH 2433	ICPH 3762	MAL 12	SIPS 1		
		ICPH 3467	ICPH 4304	SGBS-3	VL-arhar 1		
	Germplasm	ICP 11106	ICP 11443	ICP 12026	ICP 12792	ICP 7349	
		ICP 11120	ICP 11447	ICP 12728	ICP 13402		
		ICP 11153	ICP 1202	ICP 12751	ICP 3782		
	Susceptible (<25)	Elite inbred lines	ICPA 2043	ICPL 20212	ICPL 20227	ICPL 89	ICPL 93107
			ICPB 2043	ICPL 20213	ICPL 20230	ICPL 90048	ICPL 96053
ICPL 12747			ICPL 20215	ICPL 20231	ICPL 91032	ICPL 98011	
ICPL 12761			ICPL 20216	ICPL 81-9	ICPL 92010	ICPL 98013	
ICPL 149			ICPL 20221	ICPL 86022	ICPL 92041	ICPL 99044	
ICPL 20			ICPL 20223	ICPL 87	ICPL 92067		
ICPL 20210			ICPL 20225	ICPL 88034	ICPL 93017		
Hybrids and Varieties		BDN 2	ICPH 3310	Kanchen	SIPS 6	GAUT 90-1	
		BRG 3	ICPH 4305	<u>SIPS 2</u>	SIPS 7		
		HPL 24	<u>Kamica</u>	SIPS 4	SIPS 8		
Germplasm		ICP 11100	ICP 11681	ICP 12749	ICP 13581	ICP 9320	
		ICP 11145	ICP 12714	ICP 12780	ICP 2131	ICP 9801	
		ICP 11149				ICP 7035	

465

466

467 #Genotypes in italic and bold showed consistent higher survival rate after the *in vitro*, pot and
 468 field evaluations, while genotypes underlined and bold showed susceptible reaction for water-
 469 logging tolerance across screenings.

470

471

472

473

474

475

476

477

478

479

480

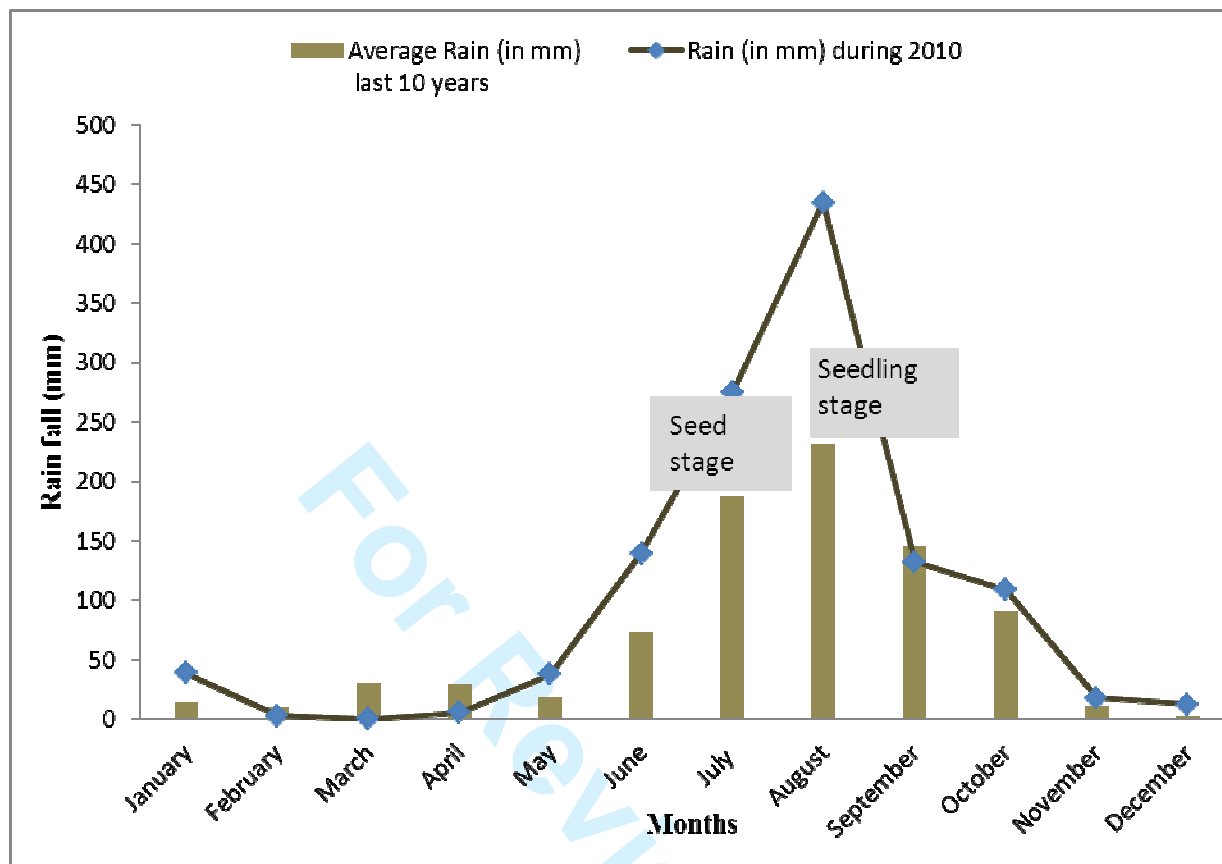


Fig. 1:

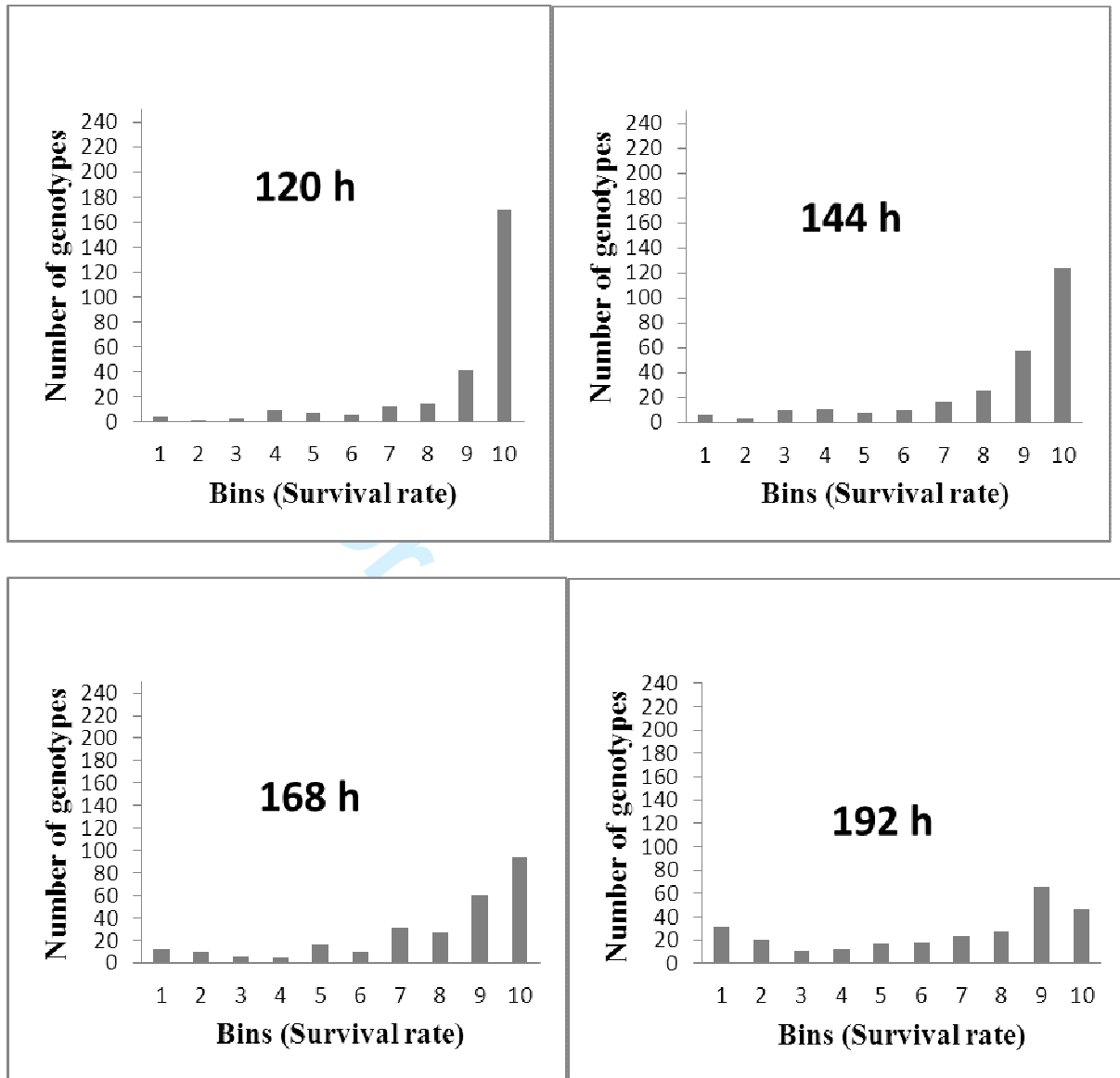


Fig. 2:

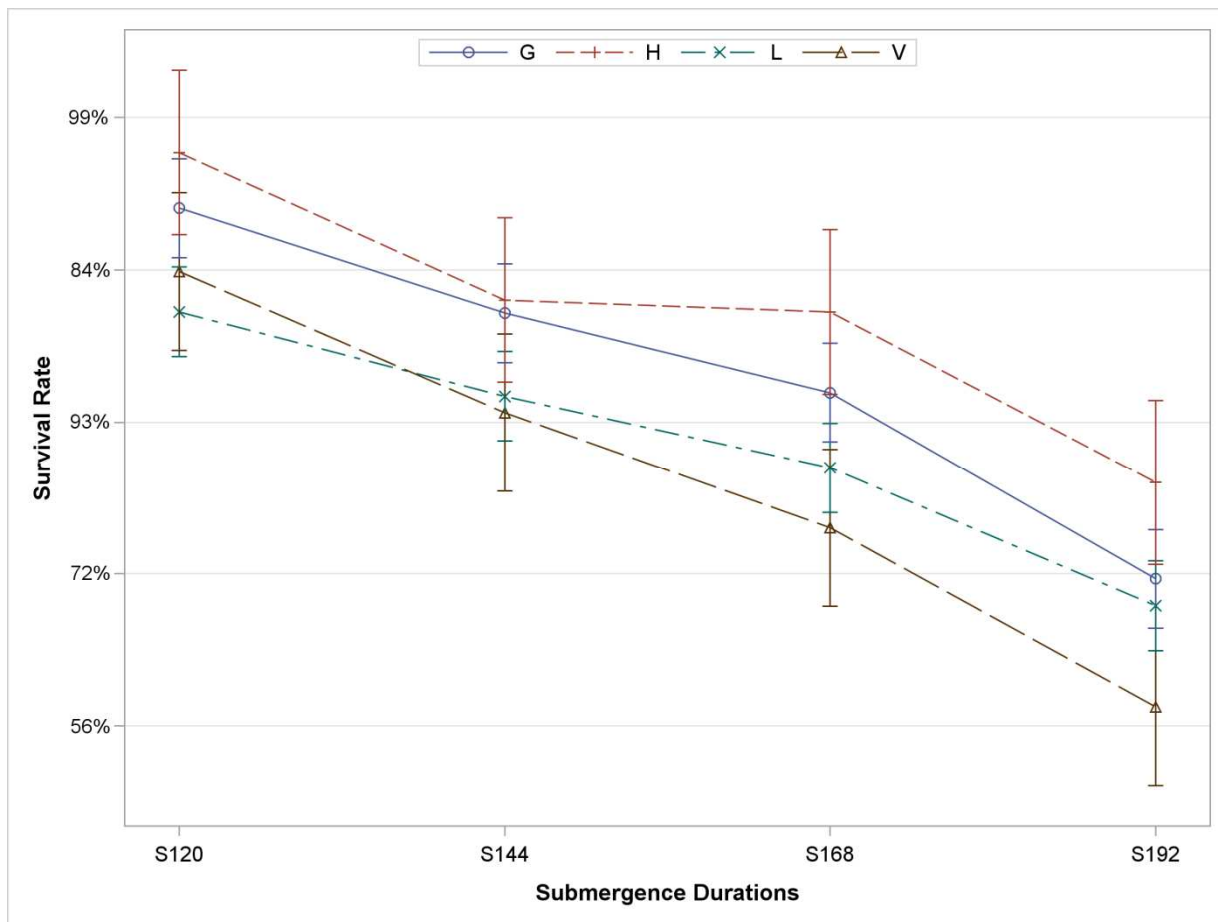


Fig.3:

W Only

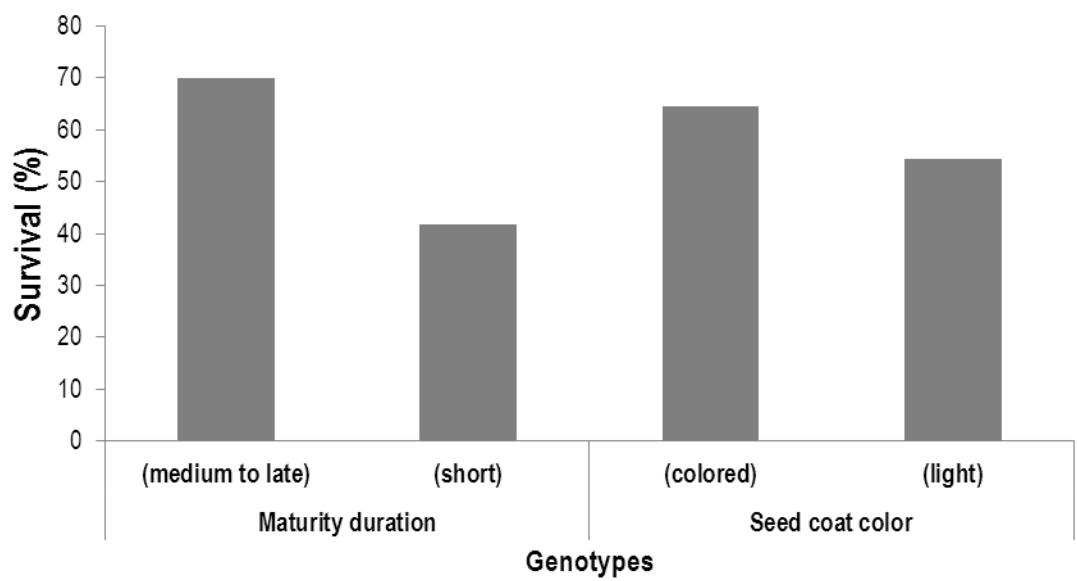


Fig. 4:

Review Only

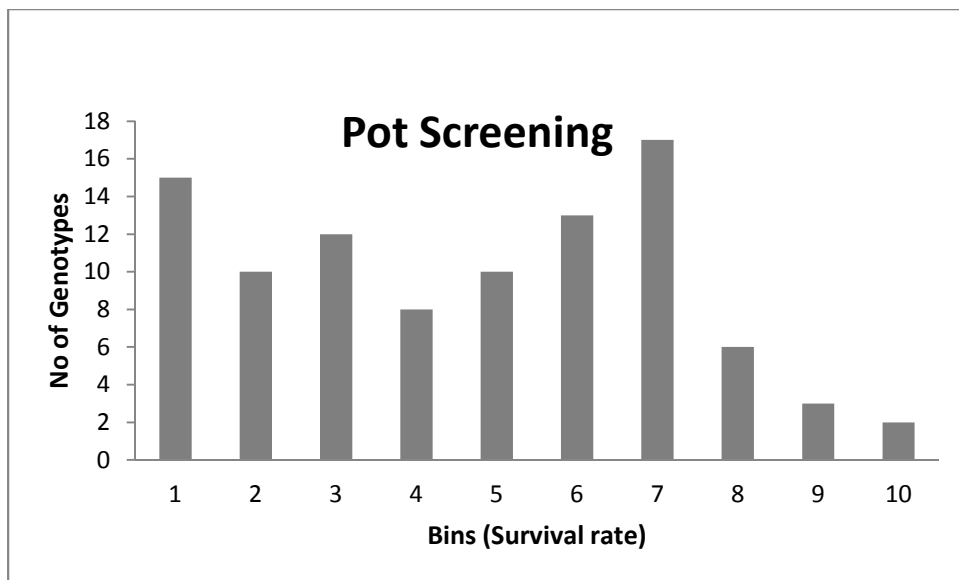


Fig.5:

For Review Only