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Germination ecology of wrinkle grass (*Ischaemum rugosum*) population of Indo-Gangetic plain region

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ABSTRACT

Wrinkle grass (*Ischaemum rugosum* Salisb.) is a highly competitive weed in rice production that can cause huge yield reductions. Information on germination ecology of this weed is essential for the development of effective integrated weed management systems. No information is available on germination ecology for populations of this weed from Indo-Gangetic plain region of India. This study was conducted to generate information on effects of major environmental factors, *viz*. light, temperature, burial depth, moisture, salinity and pH on germination/emergence of this weed. Seed germination was independent of light and more than 50% germination was recorded under a wide temperature range of 20/10 to 35/25 °C day/night (12/12 h). Maximum emergence was observed when seeds were placed on surface or buried up to 1 cm; but considerable emergence was also observed from deeper soil layers (up to 6 cm depth). Germination was above 75% at 160 mM but completely inhibited at 320 mM of NaCl. Germination was sensitive to moisture stress and was completely inhibited at water potential of -0.8 MPa. Seeds were able to germinate under wide pH range of 3-10.

Key words: Burial depth, Germination, *Ischaemum rugosum*, Light, pH, Salinity, Temperature

Weed infestations are the major biotic constraints in the rice production. Globally, weeds are estimated to account for 32% potential and 9% actual yield losses in rice (Oerke and Dehne 2004). Depending on ecological and climatic conditions, weeds can reduce rice yields by 45-95% (Manandhar *et al.* 2007). Yield reductions due to weed competition vary under different rice establishment systems. Singh *et al.* (2005) estimated 76, 71 and 63% rice yield reduction due to weeds in dry-seeded, wet-seeded and transplanted rice, respectively. Herbicides have been the major tool for weed control in rice in Indo-Gangetic plains region (IGPR) particularly in north western India. Even after herbicide applications, weeds can decrease rice yield by 10-30% (Hossain *et al.* 2016). Escaped weed plants need to be removed by manual labour, which is impractical owing to labour scarcity and rising labour wages in the region. Continuous use of herbicides with similar mode of action has resulted in the evolution of herbicide resistance in barnyard grass (*Echinochloa crus-galli*) and wrinkle grass (Iwakami *et al.* 2015, Plaza and Hernandez 2014). To counter these problems, it is necessary to develop sustainable weed management systems that may reduce both herbicide dependency and the burden of manual weeding.

Wrinkle grass (*Ischaemum rugosum* Salisb.) is an annual grass weed that is native to tropical Asia but has invaded rice fields in South Asia and Africa (Holm *et al.* 1977). At its vegetative stage, this weed resembles the rice plant, making it difficult to distinguish (Ampong-Nyarko and De Datta 1991). It is a highly competitive weed in rice production and can cause up to 48% yield reductions (Lim *et al.* 2015). Limited herbicide options like anilophos as pre- and fenoxaprop and cyhalofop as post-emergence are available for its control in rice (Duary *et al.* 2015). Many of the commonly used herbicides in rice like butachlor, pendimethalin, pretilachlor, pyrazosulfuron and oxadiargyl provide poor control of this weed (Anonymous 2015). Due to labour and water scarcity, IGPR farmers are shifting from puddled transplanted rice (PTR) to dry-direct seeded rice (DSR). In PTR, ponding of water serves as an effective weed control measure against emergence of wrinkle grass, but this weed control tool is missing in DSR resulting in more weed competitiveness (Lim *et al.* 2015). Continuous depletion of ground water in IGPR is further complicating the weed problem in PTR, as it becomes difficult to obtain enough water resulting in aerobic weeds like wrinkle grass appearing in PTR fields (Bhatt *et al.* 2016).

Information on germination ecology may identify the weakest link in the life-history of a weed which is essential for the identification and development of effective integrated weed management systems for better weed control (Bhowmik 1997). Seed germination is the first stage at which the weed can compete for an ecological

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niche and is mediated by various environmental variables such as temperature, light, pH, soil salinity and moisture (Chachalis and Reddy 2000). Competitiveness of any weed species in an agro-ecosystem depends on the ability of seeds to germinate in response to different environmental factors. The requirement for light is the principal means by which germination may be restricted to an area close to the soil surface and species requiring light for germination are likely to be more prevalent in no-till cropping systems (Cousens *et al.* 1993). Temperature is an important factor for successful seed germination; some weeds may germinate over wide range of temperature while others require narrow temperature range for germination (Shoab *et al.* 2012, Derakhshan and Gherekhloo 2013). Soil pH, salinity and moisture stress are other environmental factors affecting seed germination and thus distribution of various weeds. Information on germination ecology has been available for wrinkle grass populations belonging to Malaysia (Bakar and Nabi 2003) and Philippines (Lim *et al.* 2015), but no such information is available for weed populations from Indian IGPR. Knowledge of the response of wrinkle grass to environmental factors will help to bring a broader perspective for its effective management and in predicting its potential to spread. This study was conducted to enhance our understanding of the role of temperature, light, moisture, salinity, pH and soil depth in germination ecology of wrinkle grass population from Indian IGPR.

MATERIALS AND METHODS

Wrinkle grass seeds were collected from several rice fields of Punjab Agricultural University, Ludhiana, India (30°56' N and 75°52' E). Cleaned seeds were stored at room temperature in air tight plastic containers. The 1000-seed weight was 3.70 ± 0.28 g.

Germination protocol: Before initiating laboratory and field experiments, wrinkle grass seeds were tested for viability using 1% tetrazolium chloride solution (Steadman 2004) and seeds possessed more than 95% viability. Uniform-sized seeds were surface sterilized using 0.1% mercuric chloride for 2 min, followed by thorough washing with distilled water to protect against any fungal infection (ISTA 2010). Seed germination was tested in 3 replicates with 30 seeds placed evenly on Whatman No.1 filter paper in 9-cm petri dishes. For studying the effect of light, moisture stress, salinity and pH, petri dishes were moistened with 5 ml of treatment solution and incubated at 30/20 ^{o}C day/night (optimal temperature) in an environmental chamber (Model MAC MSW-127, Delhi, India). For control treatment, seeds were germinated using distilled water.

Light and temperature experiment: The germination of seeds was tested under fluctuating day/night temperatures (12/12 h), *viz.* 15/5, 20/10, 25/15, 30/20, 35/25 and 40/30 °C and two light regimes – light/dark for day/night environments (12/12 h) and continuous dark (24 h). For continuous dark treatment, Petri dishes were immediately wrapped in double layers of aluminum foil after adding water to prevent light penetration; and germination count was taken on 15th day only.

Burial depth experiment: This experiment was conducted using 25-cm plastic pots placed under field conditions during months of June to August. Soil used in pots was collected from fields which had no previous incidence of wrinkle grass. Fifty seeds were sown on the soil surface in pots and covered to a depth of 0, 1, 2, 4, 6, 8 and 10 cm. The pot surface was kept moist throughout the study period. Emergence of wrinkle grass was recorded daily for one month. One set of 3 pots was not seeded to test for presence of wrinkle grass in the field soil. This experiment was repeated thrice using four replications each time.

Moisture and salinity experiment: The germination response of seeds under different levels of simulated moisture stress (0, -0.1, -0.2, -0.4, -0.6, -0.8 and -1.0 MPa) was tested using solutions of PEG 8000 prepared according to equations of Michel and Kaufmann (1973). The germination ability of seeds under different levels of salt stress was examined using NaCl solutions of 1, 10, 20, 40, 80, 160, 240, 320, 400, 450 and 500 mM concentrations.

pH experiment: The effect of pH on seed germination was investigated using buffered solutions with pH ranging from 3 to 10 (Chachalis and Reddy 2000). A 2 mM potassium hydrogen phthalate buffer solution was adjusted to pH 3 and 4 with 1 N HCl. Buffer solutions of pH 5 and 6 were prepared using 2-mM solution of MES [2-(N-morpholino) ethane sulfonic acid] adjusted with 1 N HCl or NaOH. A 2-mM HEPES [N-(2-hydroxymethyl) piperazine-N'-(2-ethane sulfonic acid)] solution was adjusted to pH 7 or 8 and 2-mM tricine [N-tris-(hydroxymethyl) methylglycine] buffer solution was adjusted to pH 9 or 10 with 1 N NaOH. Unbuffered distilled water (pH 6.6) was used as control.

Except for continuous dark treatment, germination counts were recorded at 24-h intervals for 15 days after start of the experiment, with the criterion for germination being visible protrusion of the radicle. Germination (%) was calculated as: Percent germination= (number of seeds germinated / total number of seeds sown) × 100.

Speed of germination (germination index, GI) was calculated as described by the Association of Official Seed Analysts (1983) using the following formula:

$$\mathrm{GI} = \frac{\text{No. of germinated seeds}}{\text{Days of first count}} + \cdots\cdots + \frac{\text{No. of germinated seeds}}{\text{Days of final count}}$$

Seedling vigor index (SVI) was calculated using the formula given by Abdul Baki and Anderson (1973):

Seedling vigor Index (I) = seedling length (cm) x germination (%)

Statistical analysis: All experiments were repeated three times in a completely randomized design. There were no significant differences among the results of repeated experiments, so data were pooled and analyzed (ANOVA) using statistical analysis software version 9.2 (SAS 2009). Means were separated at $\alpha \leq 0.05$ using Fisher's Protected Least Significant Difference (LSD) test (Cochran and Cox 1957).

RESULTS AND DISCUSSION

Effect of light

Wrinkle grass germinated under complete darkness indicating that germination of this weed was independent of light. The seedlings that germinated under complete darkness were etiolated having elongated and chlorotic shoots. Seed germination response to light may vary considerably from species to species. Seeds of some species like Chinese sprangletop (*Leptochloa chinensis*), rice flatsedge (*Cyperus iria*) and grass like fimbry (*Fimbristylis miliacea*) have an absolute requirement of light to germinate (Chauhan and Johnson 2008, 2009a) while for others like natalgrass (*Melinis repens*), light is not a pre-requisite for the germination (Strokes *et al.* 2011). Light is an important ecological determinant for germination, and the absence of light acts as a soil depth indicator that prevents germination of many weed species (Crisraudo *et al.* 2007). However, biotypes of a species in different geographical regions may vary in their response to light, as in the present study germination of this wrinkle grass population from IGPR was independent of light but Malaysia and Philippine populations did not germinate under complete darkness ((Bakar and Nabi 2003, Lim *et al.* 2015).

Effect of temperature

Wrinkle grass seeds of this IGPR population germinated best in the temperature regime of 30/20°C day/night (**Table 1**); however, seeds were able to germinate under a wide temperature range from15/5 to 35/25°C. The highest value of germination index (important indicator of germination speed) was recorded in diurnal temperature regime of 30/20°C day/night followed by 35/25°C. Seedlings grown at 30/20°C also exhibited greatest seedling vigor index indicating their higher competitiveness than seedlings grown at other temperatures. The weed did not germinate at 40/30°C. Temperature is an environmental factor that can influence emergence patterns and timing of seed germination (Derakhshan and Gherekhloo 2013). In IGPR, direct-seeded rice is planted in the months of May-June when mean day and night time temperature lies between 25-35°C (Gill and Kingra 2011), so emergence of wrinkle grass may occur along with rice seedlings and thus may compete during early establishment period of rice. Temperature during the months of July-August also ranges between 30-35°C and moisture also is not likely to be a constraint both in DSR and PTR situations. Therefore emergence of many flushes of this weed may be expected during the rice season in IGPR owing to its ability to germinate in wide temperature range of 15/5 to 35/25°C day/night.

Effect of burial depth

Wrinkle grass seeds exhibited maximum emergence at 0-1 cm depth (**Figure 1**). At 4 cm depth, emergence was reduced by about 50% compared to surface placed seeds. Emergence was 22% when placed at 6 cm depth and no emergence was recorded when seeds were buried at 8 cm. Apparently, this weed can germinate from deeper soil layers as its germination can take place in complete absence of light. This IGPR population exhibited 66.5% emergence when seeds were buried at 2 cm depth. In contrast to our results, the germination of wrinkle grass population of Philippines was

Table 1. Effect of temperature on germination and seedling growth of wrinkle grass

Temperature (°C) Day/night (12/12 h)	Germination (%)	Germination index	Seedling vigor index (I)
15/5	20	0.32	99.7
20/10	60	1.8	673.8
25/15	82.2	4.0	1071.5
30/20	95.5	6.1	1547.4
35/25	80.0	4.4	1145.8
40/30	0	-	-
LSD (p=0.05)	9.52	0.29	66.5

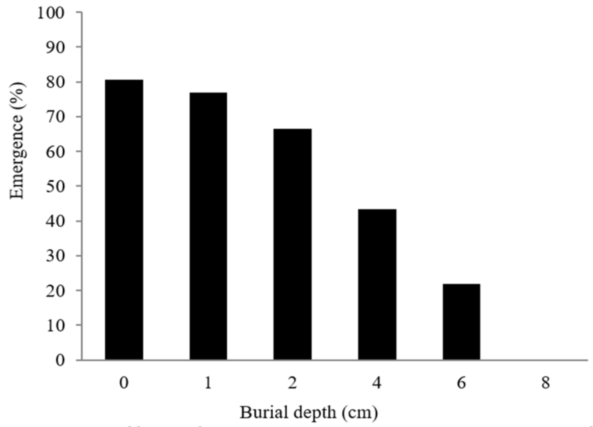

Figure 1. Effect of burial depth on emergence (%) of wrinkle grass

completely inhibited at a depth of 2 cm (Lim *et al.* 2015) indicating differential germination requirements of different populations of this weed. The requirement of light and limited availability of storage reserves are two major constraints for reduced emergence of weeds from deeper soil layers (John Bullied *et al.* 2012). In case of our population, germination was independent of light and owing to the bigger seed size of this population (1000 seed weight is 3.70 g); this weed emerged even when buried deep in the soil profile. It may be that Philippine populations have light requirements for germination or it had limited seed reserves or both (information regarding seed size of Philippines population is not available with us), factors which inhibited seed emergence from deeper soil layers. Wrinkle grass population of IGPR can germinate from deeper soil layers unlike other grass weeds of rice such as Chinese sprangletop (*Leptochloa chinensis*) which cannot emerge from soil layers deeper than 0.5 cm (Chauhan and Johnson 2008), and crowfoot grass (*Dactyloctenium aegyptium*) that exhibits only 10% emergence at 4 cm depth (Chauhan 2011). These findings indicate that emergence of this weed is not influenced by different tillage practices practiced in the IGPR and this plant can become problematic both under conventional and zero tillage systems.

Effect of salinity

Salinity stress reduced germination, germination speed and seedling vigor index starting at 10 mM NaCl (**Table 2**). Germination was completely inhibited at 320 mM of NaCl. The NaCl concentration required for 50% inhibition of maximum germination was 168 mM (**Figure 2**). This IGPR population of wrinkle grass exhibited 75.5% germination at 160 mM NaCl in contrast to Philippines population of this weed whose germination was completely inhibited at 150 mM NaCl indicating differential salt tolerance of

Table 2. Effect of sodium chloride on seed germination and seedling growth of wrinkle grass

Sodium chloride (mM)	Germination (%)	Germination index	Seedling vigor index I
10	92.2	11.8	758.9
20	87.8	11.4	640.5
40	86.1	9.63	613.5
80	77.8	9.03	554.9
160	75.5	7.00	392.3
240	17.8	0.565	40.2
320	0	0	0
Control	98.9	13.4	923.5
LSD (p=0.05)	4.73	0.881	89.7

different biotypes of same weed (Lim *et al.* 2015). Considering the seed production potential of this weed which is about 4000 seeds per plant (Holm *et al.* 1977) and ability of this population to exhibit 18% germination even at 240 mM NaCl, it can become a problematic weed even in salinity affected areas of the state. Many other weeds of rice-wheat cropping systems like junglerice (*Echinochloa colona*), common purslane (*Portulaca oleracea*) and little seed canary grass (*Phalaris minor*) have also been reported to be salt tolerant and can germinate when exposed to NaCl concentrations higher than 100 mM (Chauhan and Johnson 2009b, 2009c, Sethi and Kaur 2016). This shows that many of the weeds can germinate and grow under adverse environmental conditions where crops may have to encounter not only harsh environmental conditions but also intense weed competition.

Effect of moisture stress

Germination of wrinkle grass was sensitive to moisture stress with complete inhibition at a water potential of -0.8 MPa (**Table 3**). At -0.4 MPa,

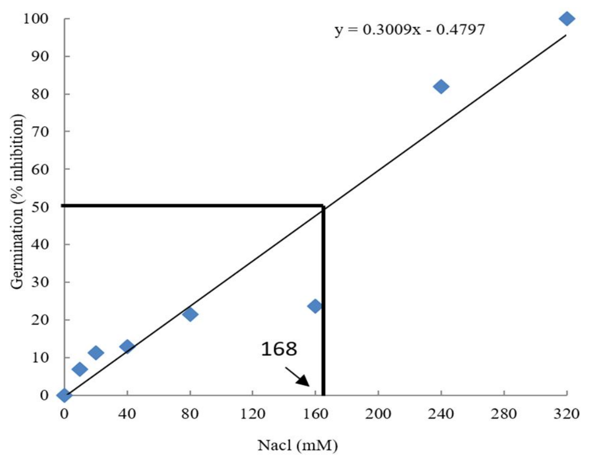

Figure 2. Effect of NaCl on germination of wrinkle grass. The NaCl concentration (168 mM) required to inhibit 50% of maximum germination is shown by an arrow

Table 3. Effect of moisture stress on seed germination and seedling growth of wrinkle grass

Osmotic potential (MPa)	Germination (%)	Germination index	Seedling vigor index (I)
-0.1	86.7	11.0	682.3
-0.2	76.7	8.09	562.3
-0.4	63.3	6.13	394.5
-0.6	57.8	4.47	271.5
-0.8	0	0	0
Control	96.7	12.9	963.8
LSD (p=0.05)	4.97	0.872	66.8

germination was reduced by 33.4% with germination speed reduced to half and seedling vigor reduced by more than half as compared to their respective controls. Like this IGPR wrinkle grass population, germination of Philippine populations of this weed were also completely inhibited at -0.8 MPa. Wrinkle grass populations of Philippines exhibited 31.5, 3.5 and 3.5% compared to 86.7, 63.3 and 57.8% germination of this IGPR population at -0.1, -0.4 and -0.6 MPa, respectively (Lim *et al.* 2015). Germination of major paddy weeds like crowfoot grass and junglerice has also been reported to be very sensitive to water stress which exhibit complete germination inhibition and only 1% germination at -0.8 MPa, respectively (Chauhan and Johnson 2009b, Chauhan 2011).

Effect of pH

The seeds of wrinkle grass were able to germinate over a pH range of 3-10. Germination of wrinkle grass at neutral pH buffer was at par with control (distilled water having pH 6.6) and it was 85.5%. Although, there was a decrease in germination with either increases or decreases in pH, wrinkle grass was able to maintain more than 50% germination under wide pH range of 3-10 except at pH 3, where it exhibited 47% germination (**Table 4**). Acidic pH proved to be more detrimental to seedling growth and vigor as compared to alkaline pH. Germination speed and seedling vigor index were maximum in control and minimum at pH 3. At pH 3, seedling vigor was reduced by 98% as compared to their control and only emergence of radicle without any shoot was exhibited by wrinkle grass seedlings. Ability of wrinkle grass to germinate under wide pH range of 3-10 indicates that this weed can become problematic in soils having extremes of pH. Germination of some weeds like slender amaranth (*Amaranthus viridis*) and coffee senna (*Cassia occidentalis*) is very sensitive to pH and can germinate only within a narrow pH range (Norsworthy and Oliveira 2005, Thomas *et al.* 2006) but other weeds like turnip weed (*Rapistrum rugosum*), cadillo (*Urena lobata*) and alien weed

Table 4. Effect of pH on seed germination and seedling growth of wrinkle grass

pH	Germination (%)	Germination index	Seedling vigor index (I)
3	46.7	5.9	16.2
4	55.5	6.2	216.4
5	54.4	5.8	510.5
6	72.2	8.9	624.4
7	85.5	10.7	845.7
8	75.5	8.7	820.0
9	74.4	8.0	637.3
10	67.7	7.7	580.2
Control (6.6)	90.4	12.3	880.0
LSD (p=0.05)	5.63	1.86	105.2

(*Tridax procumbens*) possess the ability to germinate under wide pH range from pH 4-10 (Chauhan *et al.* 2006, Wang *et al.* 2009, Vanijajiva 2014).

Seed germination is one of the most critical life events for the success of any plant species because it represents the first stage at which they can compete for an ecological niche. Germination is mediated by various environmental variables such as temperature, light, pH, soil salinity and moisture (Chachalis and Reddy 2000). Germination of the IGPR wrinkle grass population is independent of light which enables this weed to emerge not only from surface but also from deeper soil layers. Germination of this weed is sensitive to moisture stress but can occur in a wide temperature range of 20/10 to 35/25°C day/night (12/12 h), so it can germinate whenever it will encounter favorable moisture conditions in rice season of IGPR. This weed is able to germinate under high salinity conditions and wide pH range of 3-10 indicating that it may emerge as a very competitive weed even in saline, acidic and alkaline soils. Because of its high seed production potential (4000 seeds per plant), extra care should be taken by those farmers who have shifted or are shifting towards direct-seeded rice to remove this plant before seed production in order to prevent the buildup of soil seed bank of this weed. Alternatively using a stale seed bed technique before crop establishment might help in exhausting the existing seed bank of wrinkle grass.

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