INTRODUCTION

Rice (Oryza sativa L.) is one of the most important staple food crops for major population of the world (Nutan et al., 2020; Seck, Diagne, Mohanty, & Wopereis, 2012; Zhang et al., 2011). Asia accounts for 90% of global rice production and consumption (Adjao & Staatz, 2015). Rice has become closely associated with food security as well as political stability in Asian countries (Muthayya, Sugimoto, Montgomery, & Maberly, 2014). As rice crop is the backbone of livelihood of the rural population, and consumption increases with the population there is a need to encourage production by using stress-tolerant and high yielding varieties (Mahajan, Kumar, & Chauhan, 2017).

Rice is grown by farmers under rain-fed and irrigated conditions. Lacking availability of standing water at the time of seedling plantation and water stress during growth are the major factors limiting the production of rice (Zhang et al., 2018). Studies show that the increasing trends of temperature and, uncertainties and reduced amounts of rainfall are associated with increased adverse impacts on rice directly or indirectly (Rahman, Kang, Nagabhatla, & Macnee, 2017; Teixeira, Fischer, van Velthuizen, Walter, & Ewert, 2013). These events of climate change have increased frequency, duration and severity of drought and have ultimately reduced rice productivity (Allen et al., 2010; Auffhammer, Ramanathan, & Vincent, 2012; Thapa, Thapa, & Magar, 2015; Uga et al., 2013). It is expected that the drought will be severer and widespread in the coming years due to the decreasing trend of precipitation and increased evaporation (Dai, 2013). Hence, there is a need to explore drought-tolerant varieties and improve their quality for sustainable production in water-limiting or drought-prone environments.

ARTICLE INFO

Keywords:
Indigenous rice
Drought
Proline
Survival probability
Seedling survival

Article History:
Received: January 24, 2020
Accepted: June 10, 2020

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ABSTRACT

Water stress is one of the adverse factors affecting growth, development and productivity of rice. It is crucial to explore the drought tolerant rice varieties and improve their quality for sustainable production for drought-prone environments. The aim of this study was to know the ability of selected landraces (Aapjhutta, Kartika, Aanadi and Jhapamansuli) and improved varieties (Khumal-8, Khumal-10, Khumal-11 and Chainung-242) of rice to tolerate water-deficit stress in Nepal. The rice plants were grown in polyethylene pots. The pots were watered for the first two days of seedling transplantation and then watering was stopped. Survival and survival probability of seedlings were calculated. In addition, the concentration of an osmolyte (proline) was estimated after complete death of the plants in each variety. Two rice varieties, Jhapamansuli and Aapjhutta showed the highest seedling survival under water-deficit stress than the improved and other rice varieties. Results indicated that these two varieties have the ability to survive better than others under the stress by accumulating a high amount of proline as a compatible solute. Thus, these varieties can be preserved and utilized for breeding activities to develop drought tolerant and high yielding varieties.

ISSN: 0126-0537 Accredited First Grade by Ministry of Research, Technology and Higher Education of The Republic of Indonesia, Decree No: 30/E/KPT/2018

Three varieties of rice landraces Aapjhutta, Kartika, and Aanadi are the most popular varieties in the Gorkha district of Nepal. Aapjhutta is the most popular rice landrace because of its small grain size and good aroma (Joshi, 2017). Kartika is cultivated in the areas with limited water availability. Aanadi is red rice, one of the endangered rice landraces in Nepal and commonly known as the festival rice having medicinal value as well (Joshi, 2017). Rice named Jhapamansuli is also very popular in the district which was introduced about 20 years back by the local farmers but from where it was introduced is unknown. Based on seed characteristics, according to the local people and District Agriculture Office of Gorkha district, Jhapamansuli is a different variety than the improved Mansuli variety such as Samba-Mansuli. It is popular among the local farmers due to having its good productivity and taste. The local farmers have cultivated this variety near the water source (locally called as Dhaap) and therefore, it is also named as Dhapamansuli.

Introduction of improved varieties of rice has been replacing the local landraces throughout Nepal. Four of the improved varieties (Khumal-8, Khumal-10, Khumal-11, and Chainung-242) are the most popular varieties in the country. Khumal-8 was the improved variety from local landrace Jumli-Marsi and exotic genotype IR-36 in 2006 and Khumal-10 from IR-36 and Khumal-5 (Pokhreli Masino/Ku-IB-361-BLR-2-6) in 2011 (Devkota, Acharya, & Pokhrel, 2016; Upreti, 2016). The variety Khumal-11 was released in 2002 and recommended for Kathmandu valley, Nepal. The origin of Chainung-242 is the Taiwan which was released in 1966 and recommended for mid-hills of Nepal. All of these improved varieties are recommended for warm temperate regions of Nepal (Devkota, Acharya, & Pokhrel, 2016).

Characterization of phenological, morphological and physiological traits related to the water stress of such landraces by comparing with improved varieties could be one of the essential parts of plant breeding (Serraj et al., 2011). As one of the important physiological traits, plants accumulate organic and inorganic solutes in response to stressed environment (Ashraf, Akram, Al-Qurainy, & Foolad, 2011; Blum, 2017). For example, proline accumulation is an indicator of osmotic tolerance in many plant species (Kavi Kishor & Sreenivasulu, 2014; Mwadzingeni, Shimelis, Tesfay, & Tsilo, 2016; Zegaoui et al., 2017). Rice is known to have the ability to accumulate proline under drought stress (Lum, Hanafi, Rafii, & Akmar, 2014; Pandey & Shukla, 2015; Xiong et al., 2012) but the ability of accumulation may depend on varieties within species. Hence, the evaluation of proline concentration in different rice varieties can give valuable information on their stress tolerance ability.

This research aimed to test water-deficit stress on the selected rice landraces and improved varieties of Nepal so that it could give information on drought-tolerance abilities. The study selected the popularly growing rice landraces in Gorkha district of Western Nepal and compared their survival under water-deficit stress with the popular improved varieties which have contributed important food of Nepalese people.

MATERIALS AND METHODS

Seed Collection, Seedling Preparation and Transplantation

This study was conducted from May to July 2017 at Central Department of Botany, Tribhuvan University, Kathmandu, Nepal. The rice varieties selected for the study were Aapjhutta, Kartika, and Aanadi as the local landraces of Gorkha district of Nepal and Jhapamansuli as the introduced variety in the district from an unknown source. The improved varieties selected for the study were Khumal-8, Khumal-10, Khumal-11 and Chainung-242. The seeds of local landraces and Jhapamansuli were collected from Swara Village of Gorkha Municipality, Gorkha district of Nepal in January 2017 and seeds of improved varieties were collected from National Agriculture and Research Centre (NARC), Lalitpur, Nepal in February 2017. All the seeds collected were packed in airtight containers and stored at 5°C in the refrigerator until use.

Seedlings of all the rice varieties were grown at room temperature (26-29°C). During the process, the seeds were surface sterilized with 70% alcohol, washed and soaked in water for 24 hours. Then, the seeds were spread on wet filter paper in a tray and kept in dark. The seeds of all varieties germinated after 3rd day of soaking.

Garden soil was mixed thoroughly with cow dung in a ratio of 3:1. Polyethylene pots of size 12 cm in diameter and 12 cm in height were taken and filled with the mixture of soil and cow dung (900 g). After 7th days of soaking the seeds, seedlings of each variety having a homogeneous size (1 to 2
cm long) were transplanted to the pots prepared. A single pot consisted of 10 seedlings in three rings i.e. 6 seedlings in the outer ring, 3 in inner ring and one in center of soil surface. Each variety of rice had six replicated pots. Each pot was watered (200 ml normal water) on the day of transplantation. On the next day, 100 ml water was added in each pot and then watering was stopped to expose the plants to water-deficit stress. The pots containing seedlings were kept in a greenhouse. The temperature inside the greenhouse varied from 21 to 36°C and moisture 50-85%.

Seedling Survival and Proline Test

The plants exposed to water-deficit stress were observed daily. Numbers of seedlings survived and died in each 4-days interval were recorded. Plants were harvested after complete death. Complete death of the plants was considered when the leaves of stressed plants dried completely. The study was completed on the 7th week (49 days) after the seedling transplantation.

Proline content in each variety was estimated after death (Bates, Waldren, & Teare, 1973). Initially, 0.25 g plant material was ground with 3% sulphosalicylic acid (10 ml). The extract was centrifuged at 3000 rpm for 10 minutes. Then, 2 ml supernatant was transferred into a test tube and 2 ml 6 M orthophosphoric acid, 2 ml acid ninhydrin solution and 2 ml glacial acetic acid were added and kept in the water bath at 100°C for 1 hour. In the solution 4 ml toluene was added, shook well, and allowed to settle for a while. Then, the upper layer formed was taken in a test tube to record the observance value (OD value) using a spectrophotometer at 520 nm wavelength. Different concentrations of pure proline (20, 40, 60, 80, and 100 ppm solutions) were prepared, and OD value was recorded at the same wavelength. From the OD value of pure proline, a standard graph was plotted. The concentration of proline in the rice samples was estimated by using the standard graph and further calculated using the formula:

Amount of proline (µmole/g sample) = ([µg proline/ ml × ml toluene]/115.5 µg/µmole) / [g sample]/5

Statistical Analysis

Seedling survival data were analyzed using the Kaplan–Meier method (Kaplan & Meier, 1958) and survival curves were compared by log-rank test. The data were interval-censored because seedlings died within the study period. To analyze the data on seedlings’ weekly survival probability, repeated measures analysis of variance was applied. Proline content of seedlings among the varieties was compared using a one-way analysis of variance (ANOVA) and Tukey’s significance (Tukey’s HSD) tests. Survival probability data of days 27, 31, and 43 were not normal and hence they were analyzed by non-parametric Kruskal-Wallis test. The value of P ≤ 0.05 was considered statistically significant.

RESULTS AND DISCUSSION

Seedling Survival and Survival Probability

Seedling survival differed among all the rice varieties tested (Fig. 1, P < 0.01) but comparing survival among the improved varieties the difference was not significant (Fig. 2A, P > 0.05). Jhapamansuli showed the highest survival among the improved varieties and landraces tested (Fig. 1 and Fig. 2B).

Its survival time was 44.25 ± 0.60 days followed by Aapjhutta (41.38 ± 0.74), Chainung-242 (41.65 ± 0.73) and Khumal-10 (40.33 ± 0.78) (Table 1). Similarly, the survival time of the landrace Kartika was 40.10 ± 0.76, and the lowest survival time was found in the Anadi, Khumal-11, and Khumal-8 (39.50 ± 0.81, 39.49 ± 0.77, and 38.89 ± 0.82 days, respectively) (Table 1).

The weekly analysis showed a significant difference in seedling survival probability pattern among the rice varieties with time (P value was < 0.001 for time and time × variety). The difference in the probability started from the 4th week after seedling plantation. The improved variety Khumal-8 and landrace Anadi showed the lowest survival probability than other varieties in the 4th week. In the 5th week, the survival probability was low in Khumal-8, Kartika, and Anadi. The Jhapamansuli showed the highest survival probability i.e. 0.97 followed by Aapjhutta (0.57) in the 5th week while the least survival probability was observed in Khumal-11 i.e. 0.04 (Fig. 3).

In the 6th and 7th weeks, the survival probability of Jhapamansuli was 0.17 and 0.11 respectively. It was followed by Aapjhutta i.e. 0.12 and 0.05 respectively, while other varieties completely died after 6th week of the plantation (Fig. 3). This illustrates that the plant survival duration under water-deficit stress of the seedlings of Jhapamansuli is more than one month of exposure to the stress. All the varieties showed a quick decline in survival from
the 4th week of water-deficit stress but the declining pattern of seedling survival in Jhapamansuli started only after 5th week of the stress exposure (Fig. 3). It should be also highlighted that the seedlings of Jhapamansuli survived even under prolonged water-deficit stress until the 7th week (Survival probability 0.11). Therefore, the variety Jhapamansuli has the highest capability to tolerate the given stress among the tested varieties.

All the improved varieties (Khumal-8, Khumal-10, Khumal-11, and Chainung-242) showed similarity in survival characteristics as there was not significant differences in the survival curves (Fig. 2A) but among the landrace varieties, the Jhapamansuli had performed the best adaptability to water-deficit stress followed by Aapjhutta (Fig. 2B).

Fig. 1. Seedling survival in rice varieties based on Kaplan–Meier estimates. Differences in survival curves are significant among rice varieties (log-rank test, P < 0.01)

Table 1. Survival time estimate of rice varieties

<table>
<thead>
<tr>
<th>Rice Varieties</th>
<th>Mean for survival time (Days)</th>
<th>Std. Error</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Estimate</td>
<td>Std. Error</td>
</tr>
<tr>
<td>Jhapamansuli</td>
<td>44.25</td>
<td>0.60</td>
</tr>
<tr>
<td>Aapjhutta</td>
<td>41.38</td>
<td>0.74</td>
</tr>
<tr>
<td>Chainung-242</td>
<td>40.65</td>
<td>0.73</td>
</tr>
<tr>
<td>Khumal-10</td>
<td>40.33</td>
<td>0.78</td>
</tr>
<tr>
<td>Kartika</td>
<td>40.10</td>
<td>0.76</td>
</tr>
<tr>
<td>Anadi</td>
<td>39.50</td>
<td>0.81</td>
</tr>
<tr>
<td>Khumal-11</td>
<td>39.49</td>
<td>0.77</td>
</tr>
<tr>
<td>Khumal-8</td>
<td>38.89</td>
<td>0.82</td>
</tr>
</tbody>
</table>
Fig. 2. Seedling survival in (A) improved rice varieties and (B) local landraces and Jhapamansuli based on Kaplan–Meier estimates. Differences in survival curves are significant among rice landraces (log-rank test, $P < 0.05$)
Ability of the Rice Landraces to Tolerate Water-Deficit Stress

High survival and survival probability of Jhapamansuli and Aapjhutta (Fig. 1 and Fig. 2) (Table 1) under severe water stress indicate that the local landraces have the ability to tolerate water-deficit stress (drought) comparing to the improved varieties. All the landraces (Aapjhutta, Kartika, and Aanadi) are the most popular not only in the Gorkha district but also throughout western hills of Nepal and each variety has its own quality and habitat preference. The traditional practice of farmers is to cultivate the Kartika variety in the areas with limited water availability and hence it was thought that this variety might be drought tolerant but its tolerance was found less than Jhapamansuli and Aapjhutta.

Other three varieties, Aapjhutta, Jhapamansuli, and Aanadi are rain-fed varieties. Among them, Anadi is sticky and glutinous rice found traditionally grown and one of the endangered rice landraces in Nepal. Local people believe that this rice has medicinal properties and it should be fed to women during delivery. Aapjhutta is one of the delicious rice varieties having high yield. Jhapamansuli, the popular rice in the Gorkha district was an introduced rice from unknown source about 20 years back. Local people cultivate this variety near the water source (Dhaap) and therefore, it's another local name is Dhapamansuli. Therefore, it was expected that this variety should have less tolerance to water-deficit stress. The results of our study contradicted to the expectations as the Kartika variety did not show tolerance ability while the Jhapamansuli showed tolerance to water-deficit stress. On this basis, it can be suggested that all of these traditionally cultivating rice varieties should be used to develop drought-tolerant and high yielding or medicinal rice varieties by integrating such unique characters.

The developing countries are severely affected by climate change and the climate change is the major cause of changes in amount of rainfall and rainfall pattern (Chalise & Naranpanawa, 2016; Seaman, Sawdon, Acidri, & Petty, 2014). Reduced rainfall is a cause of drought and the drought as the abiotic stress has been responsible for a reduction in crop productivity (Fita, Rodríguez-Burruezo, Boscaiu, Prohens, & Vicente, 2015; Haile et al., 2019). The rain-fed crops like rice are facing great challenges from water-deficit stress with increasing droughts (Miyan, 2015). In this situation, scientists should focus their studies to identify drought suited
rice varieties. Plant physiologists consider that drought tolerant plants have the ability to survive or grow in water-stressed conditions (Luo, 2010). Therefore, testing the survival of plants under water-stressed conditions would be the basic experiments to characterize the drought resistance abilities in plants. There are several assays to screen plants for drought tolerance such as pot methods which are often used for characterization of morphological traits under stressed conditions (Kumar et al., 2020; Luo, 2010).

Local people may be unknown or unaware to the adaptability of landrace varieties on different climatic stresses since the varieties might have faced adverse climatic conditions for generation to generation. Among various extrinsic factors that influence rice characteristics temperature is one of the leading factor (Gutaker et al., 2020). In such a way, the landrace varieties might have developed the ability to adapt in drought stressed conditions by either natural selection or mutation due to prolonged stress. One of the objectives of plant improvement is to get the characteristics that are more suitable in the adverse environmental conditions like drought or flood. Utilization of such varieties to cope with adverse climatic conditions could be a method of a kind of climate change adaptation (Nutan et al., 2020). Development of such varieties has become a popular practice as the climate has been changed worldwide and a number of drought-tolerant rice varieties have been developed with high yielding by conventional breeding (Xoconostle-Cazares, Ramirez-Ortega, Flores-Elenes, & Ruiz-Medrano, 2010). Some of the examples are: BRRI dhan56 and BRRI dhan57 are developed in Bangladesh, Sahbhagi dhan in India, IR79971-B-191-B-B and IR79971-B-227-B-B in Indonesia, Sahod Ulan varieties and Kathian 1 in the Philippines (Gregorio, Islam, Vergara, & Thirumeni, 2013).

High Proline Concentration in the Rice Landraces

There are several studies conducted to investigate the crop’s physiological mechanism to tolerate drought stress. As a biochemical response drought-stressed plants can accumulate osmolytes such as proline. The proline plays important role in osmotic adjustment and increase the ability of plants to resist cellular dehydration caused by drought (Chen & Jiang, 2010). The proline protects and preserves functional and structural integrity of enzymes under stress (Suprasanna, Nikalje, & Rai, 2016). Proline content analysis showed significant differences among the selected rice varieties (Fig. 4, P < 0.01). The varieties Jhapamansuli and Aapjhutta were rich in proline content i.e. the concentration was high (59.0 and 58.7 µmole/g, respectively) than the other varieties. The least amount of proline was found in the improved variety Khumal-8 and Chainung-242 and the local landrace Aanadi (29.09, 22.30 and 20.20 µmole/g) (Fig. 4).

This study assumed that there must be differences in the content of proline in the rice varieties as the proline accumulation in plants has been shown to correlate with drought stress tolerance. A previous study suggested that the proline accumulation in rice cultivars is an indicator of osmotic tolerance (Dien, Thu, Moe, & Yamakawa, 2019). Studies have shown that the drought-tolerant crop varieties including rice accumulate proline in high concentrations than unstressed and nontolerant varieties (Per et al., 2017; Sahoo et al., 2019; Szabados & Savoure, 2010). Aptitude of proline accumulation under stressed condition is associated with genes, for example, certain genes may overexpress to produce a higher level of proline (Kumar, Shriram, Kavi Kishor, Jawali, & Shitole, 2010). According to the results of our experiment, the variety of Jhapamansuli could have the genetics for the ability to accumulate higher content of proline than the other varieties tested. The case was also similar to the landrace Aapjhutta and therefore, this variety is another one among the stress-tolerant varieties.

Six drought-tolerant rice varieties (Sukha Dhan series 1 to 6) have been released in Nepal but they are suited for low lands to foothill regions (Marahatta, 2016; Tripathi, Mahato, Yadav, Sah, & Adhikari, 2012). Other released rice varieties for drought tolerance are Tarahara-1 (especially for drought tolerance in flowering stage), Hardinath-1, Hardinath-2, Radha-4, and Ghaiya-1 which are direct-seeded varieties (Tripathi, Mahato, Yadav, Sah, & Adhikari, 2012). Khumal-8, Khumal-10, Khumal-11, and Chainung-242 are also recommended for mid-hills as drought-tolerant varieties (Marahatta, 2016). The landraces selected in our study (Aapjhutta, Kartika, and Anadi) and Jhapamansuli represent popular varieties for the farmers in the mid-hill region of Nepal. Therefore, these varieties can be suited for the development of new drought-tolerant and high yielding varieties for the mid-hills.
The released variety Khumal-8 is the most preferred variety in western hills and Khumal-11 in Kathmandu valley of Nepal (Shrestha & Aryal, 2011). Both are recommended for warm temperate climates (Devkota, Acharya, & Pokhrel, 2016). Although both of these varieties were susceptible to water-deficit stress they can accumulate proline in higher content than Anadi variety, the amount was not significantly different from Jhapamansuli and landrace Aapjhutta (Fig. 3). Additionally, Kartika is another water-deficit stress susceptible variety having proline content similar to the Khumal-8 and Khumal-11. This result has supported that the proline is accumulated by rice plants as drought indicator but solely the protein accumulation trait cannot be considered as characteristics for drought stress-tolerance in all varieties.

Khumal-10 is the recommended improved variety to be introduced as a drought-tolerant variety in Nepal (Marahatta, 2016). In Chainung-242, the proline content was significantly lower than other improved varieties but the survival probability of the 5th week was not significantly different from the Jhapamansuli and Aapjhutta (Fig. 3). The case was also similar to the landrace Anadi. This result indicates that the ability of tolerance to water-deficit stress of Chainung-242 and Anadi is not mediated by proline concentration.

This comparative study between landraces and improved varieties of rice has characterized some important physiological traits of them to know the tolerance ability mediated by proline under water-deficit stress. Nepal has about 2500 different rice landraces but currently, growing varieties are only 157 (CDD, 2015). These landraces have been evolved due to Nepal’s diverse agro-climatic conditions in different places (Joshi, 2017). Most of the growing landraces are becoming endangered as the people are abandoning indigenous varieties in the country. The local landraces may also have greater potential to yield than improved varieties with specific traits useful for breeding (Joshi, 2017). Hence, these characteristics must be identified and such characterization would be helpful to recognize particular variety that can be used in the improvement of new varieties integrating special traits.

Fig. 4. Amount of proline content in rice varieties under water stress (The significant differences are represented by letters above error bars)
Landrace varieties in Nepal have been used by local people since a long time ago. After the introduction of improved varieties, these landraces are given less priorities by the local farmers. The improved and hybrid varieties have started replacing the local landraces due to their easy availability in the market. But farmers are unknown about the superior characteristics of the landrace varieties over improved varieties. It is, therefore, necessary to explore and identify the superior characteristics of the landraces and make farmers aware on the advantages of the landraces over improved or hybrid varieties. Hence, such landrace varieties must be protected, promoted, and prevented from being extinct. At the same time, further research should be done for development of new varieties suitable for drought-prone environments. Moreover, the rice landraces are linked to socio-cultural processes in local communities. Hence, conservation of such landraces is not only the conservation for scientific purposes but also social and cultural values are conserved.

CONCLUSION

Overall, two rice varieties, Jhapamansuli and the landrace Aapjhutta of Gorkha district of Nepal showed the highest seedling survival under water-deficit stress than other landraces and improved rice varieties. Results indicate that these varieties have developed the ability to survive better than others under the water-deficit stress. They accumulate high amount of proline as a compatible solute than others. Therefore, these varieties should be conserved and further studies are recommended to utilize these rice varieties for breeding activities to develop drought tolerant and high yielding varieties.

ACKNOWLEDGEMENT

National Agricultural Research Council (NARC), Khumaltar, Lalitpur, Nepal and Institute for Social and Environmental Transition – Nepal (ISET-Nepal) are acknowledged for providing rice seeds and financial support, respectively.

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