# Tiller Number and Flowering Ability on BC1F1 Progeny of Interspecific Hybridization in Ruzigrass (*Urochloa ruziziensis*)

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**Abstract.** The objective of this study focusing on production $BC_1F_1$ progeny of interspecific hybridization in ruzigrass (*Urochloa ruziziensis*) were to evaluate potential of pentaploid $BC_1F_1$ hybrid for seed propagation by associating with their tiller number, flowering, and shattering ability. In addition, we evaluate the function of various nitrogen dose on vegetative stages. Eight different genotypes were examined: *B. ruziziensis* 'Kennedy', *B. decumbens* 'Basilisk', pentaploid $BC_1F_1$ line derived from *B. ruziziensis* and Mulato (RM), pentaploid $BC_1F_1$ line derived from diploid *B. ruziziensis* and tetraploid *B. ruziziensis* (RR), and pentaploid $BC_1F_1$ lines derived from *B. ruziziensis* and *B. decumbens* (RD 1-4). The experimental plants treated with three level of nitrogen fertilizer (0, 4, and 8 kg/10a). Compared with parental line, RM line had high tiller number along with increasing level of nitrogen. For the flowering and shattering ability, the first lines to flowered and shattered was the pentaploid $BC_1F_1$ (RD 1-4), RR, and *B. decumbens*. Pentaploid $BC_1F_1$ lines derived from *B. ruziziensis* and *B. decumbens* (RD 1-4) had potential for seed propagation by associating with their flowering and shattering ability, while pentaploid $BC_1F_1$ line derived from *B. ruziziensis* and Mulato (RM) had the potential biomass production and yield ability by associating with its tiller number production.

## 1 Introduction

*Urochloa* (named as Brachiaria previously) is warm-season grass belongs to a small group of genera that include Urochloa, Eriochloa, and Panicum, which all have the PEP-CK (phosphoenolpyruvate carboxynase) type of C4 photosynthetic pathway. The advantages of Urochloa e.g. excellent adaptation to low-fertility acid soils were previously reported, compatible with the tropical climate, and tolerant to drought condition [1,2,3].

The new interspecific cross case in *Urochloa* genus had been reported [4]. They have conducted the interspecific crosses between diploid *U. ruziziensis* (R. Germ. & C.M. Evrard) Crinds 'Kennedy' (2n = 2x = 18) with apomictic tetraploids U. decumbent (Stapf) R. D. Webster 'Basilisk' (2n = 4x = 36) for F1 triploid hybrid. Subsequently, $BC_1F_1$ progeny is the offspring after backcrossing the F1 triploid hybrid *Urochloa* as female parent between *B. decumbens* as male parent. $BC_1F_1$ is pentaploid hybrid Urochloa. The result backcrossing triploid is producing polyploids. Interestingly, only the pentaploid plants produced seeds by self-pollination among these polyploids. This is the first report of flowering ability of apomictic pentaploid progenies from interspecific crosses in *Urochloa* genus. These new polyploids and the research information will be useful for the new breeding source.

Polyploidy has greatest effect in plants and represents a major mechanism of specification [5]. By backcrossing with apomictic plant may lead to produce superior lines hybrid. Backcrossing also can be used to improved genomic stability and pollen viability [6]. Therefore, important to figure out the characteristic of seed propagation on $BC_1F_1$ related to flowering ability. Flowering ability the lines will be important to understand the potential for continuing the lines for next *Urochloa* breeding program. Since superior genotypes can rapidly increase by seed, it is important to develop seed production in *Urochloa* hybrid and afterward investigate characteristic.

Therefore, the objectives of this study focusing on the production on $BC_1F_1$ progeny of interspecific hybridization in ruzigrass (*Urochloa ruziziensis*) were to evaluate potential of pentaploid $BC_1F_1$ hybrids for seed propagation by associating with their flowering ability, and 2) evaluate the function of various nitrogen dose application on tiller number of *Urochloa* hybrids.

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## 2 Methodology

### 2.1 Plant material

In this study, eight different genotypes were examined: two commercial cultivar *B. ruziziensis* 'Kennedy' and *B. decumbens* 'Basilisk' as a control, four $BC_1F_1$ lines derived from the diploid *B. ruziziensis* x tetraploid *B. decumbens* which is produce $F_1$ triploid hybrid. Afterward, F1 triploid hybrids backcrossing to tetraploid *B. decumbens* as male parents and written as RD 1-4. Two $BC_1F_1$ lines derived from the diploid *B. ruziziensis* x tetraploid *B. ruziziensis* and tetraploid Hybrid Mulato which is produce $F_1$ triploid hybrid. Afterward, each F1 triploid hybrids backcrossing to tetraploid *B. decumbens* and Hybrid Mulato as male parents and written as RR and RM, respectively [4].

### 2.2 Field experiment

Seeds were first sow in nursery pots (200 $cm^2$ surface area) with vermiculate and kept in an incubator (31°C, 24 h photoperiod) for germination. After 1 week of sowing, the germinated seedlings were grown to the same pot for 15 days. The field study was conducted in Sumiyoshi Livestock Science Station, Faculty of Agriculture, University of Miyazaki, Miyazaki prefecture which located in Southern part of Kyushu, Japan (39°59′N, 131° 28′E, the elevation of 12 m above sea level). The soil type was characterized as sandy soil. The climate of Miyazaki prefecture according to the Köppen classification, is Cfa that is humid subtropical climate, relatively high temperature and evenly distributed precipitation throughout the year. The experimental plant was treated with three levels of nitrogen fertilizer 0, 4 and 8 kg N $10a^{-1}$. The experiment was laid out in randomized complete block design with three replications. All lines were fertilized with 4 and 8 kg N $10a^{-1}$ and applied at the beginning of vegetative stage.

### 2.3 Investigation on agronomy

To evaluate effect of nitrogen during vegetative stage of progenies, tiller numbers in each plot were counted manually within one week. Investigate of flowering time and shattering time were done right after finishing vegetative stage. Measured the shattering time, seed trapper has been made by nylon bag and apply in every plot. For the flowering time, we made sign at three tested stems as replication by yellow strip from the begin of heading and end of shattering.

### 2.4 Statistical analysis

Statistical analysis was conducted to compare the tiller number among plant materials and three treated nitrogen fertilizer doses, flowering, and shattering ability among plant materials. Differences in means were evaluated by Tukey's test using R statistic program.

## 3 Result and Discussion

The tiller development among the lines described the similar trend. However, there was significant difference in the genotype of *Urochloa* for tiller number. Compared with parental line (*B. decumbens* and *B. ruziziensis*), RM line had high tiller number along with increasing level of nitrogen fertilizer. Following by RR line and RD 3. Line of RD 1, 2 and 4 gave number of tillers below their parental lines without nitrogen fertilizer ($N_0$) application. Compared with $N_0$, the addition of nitrogen fertilizer ($N_4$ and $N_8$) increasing number of tillers.

**Table 1.** Tiller number among lines with different dose nitrogen fertilizer application

| Lines | Tiller Number | | |
|---|---|---|---|
| | $N_0$ | $N_4$ | $N_8$ |
| *B.decumbens* | $22^{b}$ | $73^{c}$ | 69 |
| *B.ruziziensis* | $31^{ab}$ | $135^{bc}$ | 139 |
| RD 1 | $12^{b}$ | $183^{ab}$ | 157 |
| RD 2 | $12^{b}$ | $164^{ab}$ | 167 |
| RD 3 | $34^{a}$ | $135^{bc}$ | 196 |
| RD 4 | $14^{b}$ | $135^{bc}$ | 152 |
| RR | $41^{ab}$ | $185^{ab}$ | 180 |
| RM | $57^{ab}$ | $247^{a}$ | 214 |

$N_0$, without N fertilizer application; $N_4$, 4 kg N fertilizer $10a^{-1}$; $N_8$, 8 kg N fertilizer $10a^{-1}$. Means in the same lines within a row followed by the same latter are not significantly different at 0.001 probability level.

In application of $N_4$ recorded significantly increasing tiller number in all lines. The increasing is significantly higher compared without nitrogen supplied. In other hand, tiller number was statistically similar by application 8 kg nitrogen $10a^{-1}$. Excess N fertilizer had less effect for tiller number on all lines, except *B. ruziziensis*, RD 2, RD 3 and RD 4. The greater number of tillers may lead to potential of greater yield ability. The data of tiller number revealed that lines with high number of ploidy has higher number of yield than small number of ploidy. High tiller number also associating with greater biomass production. New cultivar with high ploidy and high biomass production is potentially used as forage for livestock [7]. Even though C4 grasses has low nutrient, it can be increase by intercropping system with legume [8].

In this study, nitrogen fertilizer was applied during early stage of *Urochloa.* Nitrogen fertilizer altered the number of tiller during their vegetative stages [9]. Adequate supply $NO_3^-$ and $CO_2$ assimilation, the supply of assimilates to developing meristems is adequate to maintain their growth, so more tillers are produced and survive per plant and area, and similarly so with grains. Otherwise, less assimilate is available during early growth, fewer tillers survive, resulting in fewer ears and grains per ear and so less yield [10].

According the data of tiller number, RM, RR and RD 1 probably have greater number of seed yield regarding responses to nitrogen application to tiller number development. However, nitrogen is not the one influence of tiller number. Environmental conditions

also affect tiller development through effects on the carbohydrate supply or demand framework. In the absence of drought stress, radiation determines crop growth, whereas temperature drives development. As each tiller has only one phyllochron during which it can appear, temperature determines the duration of this period and consequently, low radiation and high temperature limit carbohydrate availability at the start of the tiller emergence period, resulting in a low frequency of appearance of early tillers [11].

Even there was difference tiller number among genotype, the reason is not clear yet that different appearance of tiller due to the difference number of chromosome or plant hormone [12]. Tetraploids are superior to diploid in terms of dry mater intake and feeding value, but they generally have lower tiller density [13]. Tiller number is positive correlation with photosynthesis and leaf area index. The number of cells per unit leaf area decreases with increasing ploidy level, therefore, even if the photosynthetic rate per cell is higher, photosynthetic rate per unit leaf might be equal to or lower than those of diploids [14].

**Table 2.** Flowering time of $BC_1F_1$ progeny and Uruchloa cultivars

| Lines | Flowering | | |
|---|---|---|---|
| | Begin | Half | End |
| *B. decumbens* | 1 Sep (10) | 11 Sep (10) | 25 Sep |
| *B. ruziziensis* | 2 Nov (11) | 13 Nov (11) | 24 Nov |
| RD 1 | 1 Sep (10) | 11 Sep (10) | 25 Sep |
| RD 2 | 1 Sep (10) | 11 Sep (10) | 25 Sep |
| RD 3 | 1 Sep (10) | 11 Sep (10) | 25 Sep |
| RD 4 | 1 Sep (10) | 11 Sep (10) | 25 Sep |
| RR | 1 Sep (10) | 11 Sep (10) | 25 Sep |
| RM | 8 Sep (12) | 22 Sep (12) | 5 Oct |

*(n): days between stage to stage

The beginning of flowering stage was difference in each lines. Among the 8 lines observed in flowering time, the first lines to flower was the pentaploid $BC_1F_1$ RD1-4, pentaploid $BC_1F_1$ RR and *B. decumbens* on September 1. The last line to begin flowering was the *B. ruziziensis* on November 2. Based on Table 2. the pentaploid $BC_1F_1$ RD1-4, pentaploid $BC_1F_1$ RR and *B. decumbens* were fully flowering on September 25. Following with pentaploid $BC_1F_1$ RM and *B. ruziziensis* on October 5 and November 24, respectively. The total period of flowering stage for all lines (from heading to fully flowering) was 20-24 d.

Flowering and shattering stage were effect by the temperature and genotype of species. Interestingly that among lines have different mode of reproduction, sexual and apomixis. *B. ruziziensis* is diploid sexual and the others are pentaploid apomixis, except *B. decumbens* is tetraploid apomixis. Apomixis *B. decumbens* very early flowering than sexual *B. ruziziensis*. Crossing between both species will be produced recombinant individuals that had a modest flowering period both the sexual and the apomictic component in interspecific hybridization is an essential condition for planning controlled crosses and achieving hybrids [15].

**Table 3.** Shattering time of BC1F1 progeny and Uruchloa cultivars

| Lines | Shattering | | |
|---|---|---|---|
| | Begin | Half | End |
| *B. decumbens* | 26 Sep (12) | 8 Oct (13) | 21 Oct |
| *B. ruziziensis* | 25 Nov (12) | 5 Dec (13) | 18 Dec |
| RD 1 | 26 Sep (12) | 8 Oct (13) | 21 Oct |
| RD 2 | 26 Sep (12) | 8 Oct (13) | 21 Oct |
| RD 3 | 26 Sep (12) | 8 Oct (13) | 21 Oct |
| RD 4 | 26 Sep (12) | 8 Oct (13) | 21 Oct |
| RR | 26 Sep (12) | 8 Oct (13) | 21 Oct |
| RM | 6 Oct (12) | 18 Oct (13) | 31 Oct |

*(n): days between stage to stage

Spikelet was shattered after finished fully flowering. The first time to shattered obviously was the pentaploid $BC_1F_1$ RD1-4, pentaploid $BC_1F_1$ RR and *B. decumbens* on September 26. The last line to begin shattered was the *B. ruziziensis* on November 25. According the data on Table 3. spikelet was fully shattered on October 21 for the pentaploid $BC_1F_1$ RD1-4, pentaploid $BC_1F_1$ RR and *B. decumbens.* The total period of spikelet for shattering in all lines was 25 d. Future research about plant size and seed size need to be done. In *Urochloa Hybrid Mulato* II, the establishment better in summer because of the greater probablity of adequate temperature and moisture condition. Spring planting may be used but manifest the risk of dry condition and stand failure [16].

In conclusion, pentaploid $BC_1F_1$ lines derived from *B. ruziziensis* and *B. decumbens* (RD 1-4) had potential for seed propagation by associating with their flowering and shattering ability, while pentaploid $BC_1F_1$ line derived from *B. ruziziensis* and Mulato (RM) had the potential biomass production and yield ability by associating with its tiller number production.

## References

1. G. Keller-Grein, B.L. Maass, J. Hanson, Natural variation in *Brachiaria* and existing germplasm collections. In: *Brachiaria*; Biology, Agronomy and Improvement (Eds Miles JW, Maass BL, do Valle CB), CIAT, Cali, 16-42 (1996)
2. N. Umami, A.H.I. Kusuma, C.T.Noviandi, Buletin Peternakan 42, 3 (2018)
3. N. Suseno, N. Isnaini, B. Suwignyo, B. Suhartanto, N. Umami, Buletin Peternakan 45, 2 (2021)
4. P. Nitthaisong, G. Ishigaki, K. Suenaga, M. Muguerza, H. Tanaka, R. Akashi, Crop Sci **59** (2019)
5. K.L. Adams, J.F. Wendel, Curr Opin Plan Biol **8**, 2 (2005)
6. R.M. Giacomin, R. Assis, S.P. Brammer, A. Nascimento Junior, P.R. Da Silva, Genet Mol Res **14**, 3 (2015)
7. G. Ishigaki, P. Nitthaisong, Y.S. Prasojo, K. Ikuo, F. Kiichi, M.M. Rohman, Anim. Bio. Sci **31**, 5 (2018)

8. Y.S. Prasojo, M. Niimi, M.M. Rahman, G. Ishigaki, R. Akashi, J. Anim. Plant. Sci **32**, 2 (2022)
9. K. Praminak, A.K.Bera, J.Agr.Plant.Prod **4**,5 (2013)
10. D.W. Lawlor, J.Exp.Bot **83**, 370 (2001)
11. H.K. Kim, E. Van Oosterom, M. Dinkhun, D. Luquet, G. Hammer, J. Ann. Bot **106** (2010)
12. Y. Liu, G. Dandan, D. Yanfeng, W. Qiangsheng, L. Ganghua, W. Shaohua Aus. J. Crop. Sci **5**, 8 (2011)
13. J.H. Neuteboom, E.A. Latinga, H. Schlepers, L. Mannetje, *Sward characteristics of a diploid and a tetraploid cultivar of perennial ryegrass as measured by different sampling techniques*, in Proceedings of the XVII International Grassland Congress, New Zealand (1993).
14. M. te Beest, J.J.L. Roux, D.M. Richardson, A.K. Brysting, J. Suda, M. Kubesova, P. Pysek, J. Ann. Bot **109** (2012)
15. R.M. Simeao, A.S. Silva, C.B. De Valle, Crop. Breed. App. Bio **16** (2016)
16. J. Vendramini, B. Seller, L.E. Sollenberger, M. Silveira, SS AGR **303** (2014)