Studies on Seed Production of *Urochloa* spp. under Different Cultivation Practices for Maximizing Seed Yield

異なる栽培条件下でのウロクロア属草種の種子生産に関する研究

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LIST OF ABBREVIATIONS

ANOVA	Analysis of variance
ANRDC	Animal Nutrition Research and Development Center
BAND	Bureau of Animal Nutrition Development
CCD	Closing cut date
CIAT	International Center for Tropical Agriculture
CRD	Completely randomized design
DLD	Department of Livestock Development
DM	Dry matter
DW	Distilled water
EC	Electric conductivity
FSP	Filled seed percentage
FTN	Fertile tiller number per square meter
FTN/P	Fertile tiller number per plant
FTP	Fertile tiller percentage
GA_3	Gibberellic acid
GP	Germination percentage
H_2SO_4	Sulfuric acid
IN	Inflorescence number per square meter
IN/P	Inflorescence number per plant
IN/T	Inflorescence number per tiller
ISTA	International Seed Testing Association
IVDMD	*in vitro* dry matter digestibility
KNO_3	Potassium nitrate
LDD	Land Development Department

LSD	Least significant difference
MAS	Marker-assisted selection
N	Nitrogen
N-rate	Rate of nitrogen application
OM	Organic matter
PGSY	Pure germinated seed yield
PP	Purity percentage
PSY	Pure seed yield
RCBD	Randomized complete block design
RH	Relative humidity
RN/I	Raceme number per inflorescence
SMC	Seed moisture content
SN/R	Spikelet number per raceme
SY	Seed yield
TN	Tiller number per square meter
TN/P	Total number of tillers per plant
TSW	Thousand seed weight
TSY	Total seed yield
TZ	Tetrazolium test
USDA	United States Department of Agriculture
WUP	Water uptake percentage

CHAPTER 1

General Introduction

Animal feed is regarded as a demand-led “livestock revolution”, which is occurring as a result of a rapidly growing population, rising incomes, and increasing urbanization. Global demand for livestock and livestock products is expected to double by 2050, with developing countries experiencing the greatest increases. The global cattle population is predicted to increase from 1.5 billion to 2.6 billion and the global goat and sheep populations from 1.7 billion to 2.7 billion between 2000 and 2050 (Turk, 2016; Cook et al., 2020).

With a rapidly growing dairy and meat industry, the demand for forage is increasing, creating a demand for quality forage seed and driving the market globally. Forage species with high yields and nutritional values, as well as ease of cultivation and management practices, are consequently required to raise livestock production. Moreover, new cultivars are urgently needed to increase pasture diversification as insurance against the extensive monoculture that has formed, as well as to provide alternative forages against feed shortages and low forage quality (Valle et al., 2013).

Sufficient quantities of forage grass seed are not available in many countries across the globe. Seed import is difficult for a number of reasons, including a limitation of appropriate species, quarantine restrictions, and high prices. Furthermore, the aftermath of COVID-19 led to serious restrictions on transportation, which impacted industries worldwide, including the international forage seed trade. Shipments were affected by lockdowns imposed by governments across the region to combat the COVID-19 pandemic (Mordor Intelligence, 2022). Reducing the use of imported seeds and increasing regional or local seed production are critical to solving these problems. They will be the key points in the future self-sufficiency of domestic livestock production.

In Thailand, the government plans to increase the quantity of meat and milk for both domestic consumption and export. Therefore, the government has already made significant efforts to develop pastureland for small farmers, who are the primary producers of meat and milk in the country. As a result, a great supply of forage seed in Thailand was required. However, there were several problems faced in forage seed production, i.e., the appropriate seed varieties, cultivation management, inspection, and the system for maintaining the quality of seed techniques were not well developed, and the seed market was limited (Japan-Thailand joint evaluation committee, 2004).

Currently, the Thai seed industry is among the most well-developed in the Asia-Pacific region. A number of organizations within the Ministry of Agriculture and Cooperatives and external agencies such as the Thai Seed Trade Association and the Seed Association of Thailand are working towards developing Thailand into a seed production hub. The main strategies for achieving this goal have been to increase seed export potential (mainly for maize and vegetables) and to provide Thai farmers with quality seeds (mainly for rice, pulses, and forage crops).

Experience in Thailand has shown that forage seed production can be an economically viable and sustainable cash crop for smallholder farmers. The future viability of the seed industry will be determined by efforts to expand into export markets as well as local sowings and the development of additional pasture species. Seed production and supply for grass species adapted to various environments and purposes will be required in the near future. As demands continue to increase, there is a need to expand the range of species grown to service a wider range of markets, e.g., high quality forage for dairy production, salt-tolerant forages, and even rehabilitation of degraded land. Consequently, regional diversity of forage seed production should have been considered.

1. Background Information

1.1 General description and importance of *Urochloa* spp.

Urochloa (synonym *Brachiaria*) grass is one of the few tropical forages, which are the main source of nutrition for ruminant livestock in tropical and subtropical areas across the globe. The genus *Urochloa* is in the Poaceae family and consists of about 100 grass species. Most of these grasses are perennial and are used as cover crops and fodder crops (Baptistella et al., 2020). It comprises important species used as forage crops, and these mainly include *Urochloa brizantha* (Hochst. ex A. Rich.) R.D. Webster (syn. *Brachiaria brizantha* Hochst. ex A. Rich), *Urochloa decumbens* (Stapf) R.D. Webster (syn. *Brachiaria decumbens* Stapf), *Urochloa ruziziensis* (R. Germ. & Evrard) Crins (syn. *Brachiaria ruziziensis* R. Germ & Evrard), and *Urochloa humidicola* (Rendle) Morrone & Zuloaga (syn. *Brachiaria humidicola* Rendle; Torres González and Morton, 2005) (Ferreira et al., 2021). These species are native to East and Central Africa and have become the most widely cultivated tropical forage grasses in Asia, the South Pacific and Australia (Clémence-Aggy et al., 2021), occupying a prominent position in the economic sector related to the seed and beef/milk markets (Njarui et al., 2021; Jank et al., 2014).

All *Urochloa* spp. can be propagated both vegetatively and by seeds. The five most important species are all perennials and are propagated by seed. They are *U. brizantha* (cvv. La Libertad, Marandu), *U. decumbens* (cv. Basilisk), *U. humidicola* (cvv. Llanero, Tully), *U. mutica* and *U. ruziziensis* (cv. Kennedy). Except for *U. ruziziensis* (cv. Kennedy), which is cross-pollinating, all others mentioned are apomicts (de Souza, 1999). There has been considerable interest in *Urochloa* grasses because they have several desirable traits that include adaptation to low fertility and acidic soil, water stress and shade tolerance, high production capacity of biomass, and the ability to withstand heavy grazing and trampling (Ferreira et al., 2021). In addition, these grasses require low production costs, thus providing the most economical alternative in ruminant feeding worldwide (Ribeiro et al., 2016). Other benefits of these grasses include that they are suitable for soil erosion control and use as cover crops, minimize nitrogen loss from soils, store atmospheric carbon dioxide in soils, improve soil health and reduce greenhouse gas emissions (especially methane and nitrous oxide), as well as support wildlife as a source of feed and habitat (Njarui et al., 2021). Aside from its environmental benefits, *Urochloa* grass can be used to generate revenue by producing and selling hay and vegetative planting materials as seeds.

1.2 Breeding improvement in *Urochloa* spp.

The interest in this genus is justified due to the genetic variability discovered for tolerance to drought, flooding, nutritional limitation, soil acidity, and several diseases (Baptistella et al., 2020). There are interesting traits in various accessions that need to be combined to obtain a hybrid with superior performance. Existing *Urochloa* breeding programs concentrate on known deficiencies in commercial cultivars and address germplasm collection as a source of desirable traits. In crosses with elite sexuals, the best genotypes should be used as apomictic progenitors. The immediate objective is to develop apomictic cultivars that combine the persistence, productivity, and adaptation to infertile acid soils of common *U. decumbens* cv. Basilisk with the durable antibiotic resistance to spittlebugs found in *U. brizantha* cv. Marandu is complemented by the high nutritive value and abundant flowering of *U. ruziziensis* (Valle, 2009).

Breeding improvement for *Urochloa* is one of the factors necessary to support the rapid and continuous development of improved tropical forage grasses to be used for feeding livestock. However, breeding programs of *Urochloa* have been delayed for a long time due to apomixes and differences in ploidy (Sweitzer et al., 2021). The more widespread use of improved varieties of forages depends upon the continuous availability of seed or planting material that is true type, free from weeds, inexpensive to obtain, and which will reliably establish good pastures when planted. However, it is known that a low seed-set rate (1–12%) significantly hindered the development of *Urochloa* hybrid breeding (Hare et al., 2015a).

Urochloa grass breeding programs have primarily been carried out by the International Center for Tropical Agriculture (CIAT) and the Brazilian Agricultural Research Corporation (EMBRAPA). A *Urochloa* breeding program initiated in 1988 at CIAT (International Center for Tropical Agriculture, Cali, Colombia), led by Dr. John W. Miles, combined desirable attributes found in accessions of *U. brizantha* and *U. decumbens*. Three apomictic hybrids have been released (cvv. 'Mulato', 'Mulato II', and 'Cayman'). Cultivar 'Mulato II' is a valuable grass for improved beef and dairy production due to its exceptional drought tolerance, year-round production of green forage, leafiness, high digestibility, and higher crude protein levels than most other tropical grasses (Pizarro et al., 2013). Cultivar 'Cayman' also exhibits many of the qualities of cv. 'Mulato II', and its distinguished waterlogging tolerance adds a new dimension to the hybrid *Urochloa* collection. In addition, cv. 'Mulato' showed agronomic potential, but seed yields were low. In many trials in Asia, Africa, and the Americas, the hybrid *Urochloa* cultivars have consistently produced significantly more dry-season forage than other tropical grasses and other *Urochloa* grasses (Cardoso et al., 2020).

Urochloa is a tropical forage grass that is well adapted to poor soils and is resistant to long dry periods (Miles et al., 2004). *Urochloa* species such as *U. brizantha*, *U. decumbens*, and *U. ruziziensis* were introduced to the subtropical and temperate regions of Japan (Kyushu and the Okinawa islands). High forage dry matter yields have been reported in some of these species in Okinawa (Hanagasaki et al., 2008). Additionally, tetraploid sexual ruzigrass was bred by *in vitro*-colchicine treatment with multiple-shoot clumps (Ishigaki et al., 2009a) or seedlings for the *Urochloa* breeding program as a source of sexuality (Ishigaki et al., 2009b). The newly bred tetraploid ruzigrass exhibited the good preference of Japanese Black cattle (Ishigaki et al., 2018). Attempts to develop a recurrent selection method among the tetraploid ruzigrass population or a hybridization method between tetraploid ruzigrass and apomictic tetraploid ruzigrasses such as *U. brizantha* and *U. decumbens* will generate new superior strains that have good drying characteristics for hay and silage production (Ishigaki et al., 2019).

Current *Urochloa* spp. breeding programs are centered on the development of new technologies capable of positively impacting livestock in tropical regions with lower resource inputs (water, energy, fertilizers, and pesticides) (Ferreira et al., 2021). Valuable traits of *Urochloa* include biomass yield, physiological tolerance to low-fertility acid soils of the tropics (Arroyave et al., 2011), digestibility and energy content (Hanley et al., 2020), insect tolerance (particularly to neotropical spittlebugs; Miles et al., 2006), and disease resistance (Valério et al., 1996; Alvarez et al., 2014; Hernandez et al., 2017). To maintain the long-term sustainability of animal and pasture production systems, new *Urochloa* cultivars (hybrid or not) need to combine adequate forage production with adaptation to soil and climate constraints. Thus, varieties should have high nutritive value and resistance to biotic and abiotic stresses, as well as high forage and seed production, to ensure that pasture is converted into valuable animal protein with added economic value (Singh, 2009).

Urochloa spp. reproduces both sexually and asexually, allowing for the production of both open-pollinated and apomictic cultivars. Recurrent selection is the most common breeding strategy for open-pollinated cultivars (Simmonds, 1979). In forage grass breeding, two types of recurrent selection are used: mass selection and polycross methods (Burson & Young, 2001). Apomixis, or asexual reproduction through seed, is critical for developing new crop varieties for widespread use. It is an important trait for breeding because it can lead to eternal asexual propagation. Crops that reproduce through apomixis retain the same traits from one generation to the next, effectively locking in desirable traits such as drought tolerance or high nutritional value. Both ecotype selection and hybridization can be used for the development of apomictic cultivars (Vogel & Burson, 2004). Many tropical forage grasses, including *Urochloa*, reproduce by apomixis (Blake, 2016). The benefit of apomixis is that when forage breeders discover an outstanding fodder crop with the right combination of desirable traits, they can faithfully reproduce it through seeds over many generations without losing that crucial hybrid vigor. Apomixis is also beneficial to smallholder farmers in developing countries who have limited resources to invest in improving their farms. Improved grass varieties that produce enough trait-retaining seeds can eliminate the need to purchase new seeds for each planting, which can be an expensive barrier to adoption (CIAT, 2019).

A breeding scheme based on recurrent selection for specific combining ability and designed to accumulate nonadditive effects is suggested as an appropriate scheme for the improvement of apomictic tropical grasses (Miles, 2007). Several successful hybrid cultivars have resulted from the careful and effective recurrent selection of tetraploid sexual lines for vigor, growth habit, leafiness, and spittlebug resistance. The hybridization breeding of *Urochloa* grasses began with the establishment of sexual tetraploid lines. Research on tetraploid sexual lines of *Urochloa* spp. has continued in tropical and subtropical Japan and Southeast Asia (Ishigaki et al., 2010; Akiyama et al., 2010). A recent breeding program for *Urochloa* spp. has also been undertaken in the tropical monsoon region, particularly in Thailand. Some progress in breeding has been made using molecular biology methods in addition to traditional methods (Ebina et al., 2005). Marker-assisted selection (MAS) for breeding populations has been previously done with some important crops and forages and was applied to *Urochloa* spp. for spawning and faster breeding.

1.3 *Urochloa* spp. seed production

The majority of tropical forages, including *Urochloa* spp., are propagated by seed. Seed production is a crucial step in the commercialization of newly released fodder plants. Most improved pastures are established from seed (Loch, 1985a). To support widespread use of current and future *Urochloa* cultivars, abundant, low-cost, high-quality seed is required

(Hopkinson et al., 1996). In addition, to be commercially viable, a forage grass cultivar must be capable of producing a seed crop with sufficient yields to be marketed at a reasonable price.

Seed yield is a major trait of interest for forage grass species, and it has gotten more attention recently because seed multiplication is economically important for novel grass cultivars to compete commercially. Seed yield is a complex trait that is influenced by genetic variation, as well as environmental factors and agricultural practices (Boelt & Studer, 2010). Success in seed production comprises four major factors, i.e., locality for growing the crop, crop management system, harvesting method, and attention to the problems of seed quality. For tropical grass seed production, an important aim of agronomic management is to produce a synchronized high-yielding seed crop by promoting inflorescence development and restricting this to a short period of emergence (Loch et al., 1999a). Optimizing agronomical practices such as establishment techniques, nitrogen application, the use of plant growth regulators, harvesting, and seed cleaning procedures can result in increased seed yield. Crop management is a key factor in determining seed yield in terms of both quantity and quality, i.e., purity and germination ability.

Cultivar 'Mulato II' [*Urochloa ruziziensis* (syn. *Brachiaria ruziziensis*) × *U. brizantha* (syn. *B. brizantha*) × *U. decumbens* (syn. *B. decumbens*)] is one of the most important *Urochloa* hybrid cultivars grown in Thailand for seed production. It was the second hybrid *Urochloa* cultivar released from the hybridization programs begun in 1988 at CIAT in Cali, Colombia (Argel et al., 2007), and it was introduced into Thailand in 2003 by Tropical Seeds LLC, a U.S. forage seed company (Hare, 2014). Despite the fact that cv. 'Mulato II' produced 60% higher seed yields than cv. 'Mulato' (*U. ruziziensis* × *U. brizantha*), the first hybrid *Urochloa* released (Hare et al., 2007a), cv. 'Mulato II' seed yields of 232–258 kg/ha were still very low when compared with yields from other commercial *Urochloa* cultivars (not hybrids). Seed yields of cv. 'Mulato II' had always been less than half of those of ruzigrass grown in Thailand.

(Kowitthayakorn & Phaikaew, 1993; Hare et al., 2007a). Even though cv. ‘Mulato II’ flowers abundantly, there is a significant failure of seed-set by seed harvest (Miles & Hare, 2007), with nearly 90% of the spikelets producing non-viable seed (Hare et al., 2014). However, because of its high forage quality, drought tolerance, and persistence, demand for cv. ‘Mulato II’ seed has rapidly increased. Nowadays, cv. ‘Mulato II’ seeds and other forage seeds produced in Thailand have created a market for export to other tropical countries in Asia and Africa. Ubon Forage Seeds Co. Ltd., a tropical forage seed company in Thailand, conducted a series of field experiments from 2004 to 2013 in an attempt to increase cv. ‘Mulato II’ seed yields through agronomic management. Field trials were conducted on planting time (Hare et al., 2007a), closing date (Hare et al., 2007b), and seed harvesting methods (Hare et al., 2007c). As a result of this research, seed yields have increased from 250 kg/ha to over 600 kg/ha. Through this research, the increasing seed yields of cv. ‘Mulato II’ can be improved by appropriate crop management.

Field trials conducted by the Thailand Department of Livestock Development (DLD) from 2004 to 2006 in Northern Thailand (18.3°N, 99°E; 320 m asl) showed that cv. ‘Mulato’ and cv. ‘Mulato II’ produced significantly higher dry matter (DM) yields than ruzigrass, but ruzigrass produced 3–4 times the seed yields of cv. ‘Mulato’ and cv. ‘Mulato II’. In the dry season, cv. ‘Mulato’ and cv. ‘Mulato II’ produced 60% more DM than ruzigrass. Cultivar ‘Mulato II’ produced more seed yields (500 kg/ha) than cv. ‘Mulato’ (220 kg/ha) and the other *Urochloa* hybrids tested in seed production trials conducted from 2007 to 2008 at the same location (Pizarro & Hare, 2014). Hare et al. (2015a) suggested that commercial seed yields from hybrid *Urochloa* must be at least 600–700 kg/ha in order to compete in price with commercial *Urochloa* cultivars from Brazil and Australia.

Nevertheless, *U. ruziziensis* cv. ‘Kennedy’ was released in Australia (1966); ‘ruzigrass’ as a common name. It was introduced to Thailand in 1968 (CPI 30623, CIAT 605, BRA

000281, ILCA 16692). The original seed was obtained from la Station Agronomique du Lac Alaotra, Madagascar in 1961, selected on the wet tropical coast of Queensland on the basis of high yields of palatable forage and ease of seed production. The cv. 'Kennedy' is still the most popular and widely-grown grass species used for seed production and pasture improvement in Thailand. It will continue to be the dominant grass planted for seed production because it is easy to establish and produces excellent seed yields. Despite superior dry-season forage yields and broader adaptability and persistence to ruzigrass, signalgrass (*U. decumbens* cv. Basilisk) has not been promoted due to seed production difficulty (Hare et al., 2005). The success of widely adopting new varieties of *Urochoa* for producing seeds will occur when the forage is accepted by livestock, as well as when commercial seed producers in Thailand can produce seeds at similar retail prices to ruzigrass. Therefore, widespread adoption of these cultivars requires that they are in demand and that they can be produced economically by increasing seed yield under good agronomic management.

1.3.1 Cultivation management practices on seed production

The ultimate goal of any farmer in seed crop production is to maximize yield per unit area. To achieve a high yield, effective crop management practices, also known as cultural practices, appeared to be critical. Proper management of seed crops results in high yields and good quality seed.

The success of forage grass seed production depends on various factors. These include cultivars, climatic conditions, and agronomic practices such as land preparation, sowing rates and time, plant spacing, closing cut date (CCD), fertilizer application, irrigation practice, pest and disease control, and weed control (Njarui et al., 2021; Brunse & Watkin, 1992).

1.3.1.1 Plant spacing and plant density

In grasses, seed yield is maximized at optimal plant density or plant spacing (Kumar et al., 2005). Consequently, the seed rate and plant spacing adopted in seed crops are extremely important. Plant spacing has a different effect on seed production in different species and even between cultivars of the same species. Appropriate plant spacing may provide a better light environment for the flowering shoot, weed control, and a more even supply of moisture and nutrients because it reduces inter-plant competition. Optimum plant density and plant spacing are influenced by plant cultivar, soil fertility, moisture supply, and age of plant stand (Humphreys & Riveros, 1986).

1.3.1.2 Closing cut date (CCD) and nitrogen (N) fertilizer application

The CCD is known to be the date of last grazing or defoliation before the forage crop is allowed to enter reproductive development. The timing of closing cuts and N fertilizer applications ensures that crops develop and mature under favorable seasonal conditions. These management practices aim to encourage the uniform development of dense inflorescence populations in order to produce synchronized, high-yielding crops. At the start of each seed crop cycle, a closing cut should be conducted, and the necessary N fertilizer applied. This combination of cutting management and N fertilizer application promotes the rapid early production of new tillers, which is the basis of a synchronized crop of inflorescences (Loch, 1980; 1985b).

In general, the main objective of defoliation management is to produce a crop of shoots of similar age at a density suitable for the environment and ready to flower under the most favorable weather conditions for the development of a good seed crop (Wongsuwan, 1999). The final defoliation or closing cut of pasture for seed production is crucial for matching expected favorable climatic conditions, whether they can minimize the risk of moisture supply with grasses not affected by day length or must be appropriately timed to correspond to this inflexible flowering behavior that restricts inflorescence production, particularly for the short-day grasses growing near the equator (Hill & Loch, 1993). An inappropriate time of closing, especially if earlier than the optimum, will result in plants growing taller and producing excessive bulk at harvest, which can either lodge the crop or interfere with inflorescence emergence and harvesting. On the other hand, delayed closing removes many of the developing reproductive apices, resulting in low inflorescence numbers and seed yields (Hare et al., 2007b).

N is considered the most important nutritional determinant of grass seed yield, and it is well known that optimum levels are required to maximize yield (Loch, 1980). The proper application of N fertilizer is a powerful tool that seed producers can use to maximize returns. The efficient use of fertilizer, which is a major control of yield, offers the best prospect for seed producers for improving income. N fertilizer application to tropical grass seed crops is almost always profitable. Negative effects occur only when a toxic level is used, other nutrients are imbalanced, environmental stresses preclude seed harvest, or seed recovery (lodging) problems occur. To promote inflorescence development for maximum yield, N is thus applied as a single dressing as soon as possible after the closing cut (Boonman, 1972). As the rate of nitrogen fertilizer application increases, there is a steep increase in yield, followed by a diminishing rate of response as maximum yield is approached; further increases in fertilizer rate cause a decrease in yield. The seed grower must consider the inherent fertility of the soil, the cultivar grown, the age of the stand, the dependability of the weather, the usefulness of split applications, and the likely effects on seed quality when predicting the most profitable level of nitrogen fertilizer to apply (Humphreys & Riveros, 1986).

1.3.2 Seed harvesting method

The *Urochloa* grass seed crops can be harvested successfully in different ways. Choosing an appropriate method for seed harvest depends firstly on the specific species, particularly its growth habit and seed structure, the synchrony of crop development and the relative amount of standing and fallen seed. Secondly, it depends on the availability of machinery or hand labor, which is related to the appropriateness of local conditions, and finally, on previous experience (Pizarro et al., 2010). Hand-harvesting methods are the most basic and logical to use where labor is plentiful and inexpensive (Loch, 1999b). It is predominant in developing countries due to the low cost and ready availability of hand labor. The hand-harvesting method is widely used in Kenya, Thailand, Brazil, and Zimbabwe, all of which produce large amounts of tropical grass seeds. Harvesting forage grass seed can be accomplished through a variety of methods. Both manual and mechanized harvesting methods have been used for the genus *Urochloa* (Pizarro et al., 2010). Grass seed harvesting in Thailand has always been carried out manually, progressing over the last three decades from single, destructive harvests to multiple, non-destructive harvests. With single, destructive harvests, crops are either hand cut with sickles and immediately threshed or cut and then sweated before threshing (Hare et al., 2007c).

In Thailand, ruzigrass is cut with sickles. The seedheads, together with about 30 cm or so of attached culm, are cut and stacked in the shade. The cut material is then allowed to sweat for 2–3 days to remove the seeds. The sweating heaps are turned daily, separating the seeds removed during this process. The threshed seeds are dried in the sun or in a well-ventilated shed before being mechanically cleaned. More intensive harvesting methods are used on ruzigrass grown by numerous smallholder farmers in Thailand. As the crop ripens, groups of adjacent seedheads are tied together into living sheaves in the field. Ripe seeds are shaken from the tied seedheads into a broad, shallow receptacle. Ruzi seeds are collected in this manner at 3–5 days intervals until seed loss from the standing crop renders further collections economically unviable. These crops are typically harvested four times over the course of two weeks. In Brazil, signalgrass (*U. decumbens*) seed can be swept up by hand from the ground during the dry season (Loch, 1992).

Hare et al. (2007c) reported that tying nylon net bags over the seedheads to collect seed produced the highest cv. 'Mulato II' seed yield, which was twice that of knocking seedheads. Sweeping cv. 'Mulato II' seed from the ground resulted in a much lower seed yield, lighter seed, and seed with lower viability than other harvesting methods, which could be attributed to ant predation or seed rotting on the ground. Ground sweeping was not successful in Thailand in research trials, but it has been used by some village smallholder farmers, where yields of over 500 kg/ha are common. In Brazil and Mexico, this method has been the predominant method of seed harvesting for *Urochloa* spp., producing up to 700 kg/ha from either manual or machine sweeping.

1.3.3 Seed dormancy breaking in *Urochloa* spp.

Seed dormancy is a phenomenon caused by species adapting to environmental conditions. This phenomenon is especially noticeable in forage seeds. Finding ways to break this dormancy, optimize seed germination and improve pasture establishment has been increasingly challenging for researchers (Alves et al., 2017). Dormant seeds are those that do not germinate despite being exposed to favorable conditions of moisture, light, temperature, oxygen, and carbon dioxide. In other words, germination is delayed (Romani et al., 2016). In the field, dormancy causes variation in seedling emergence, impeding pasture establishment and favoring weed invasion (Martins & Silva, 2001). The seeds of *Urochloa* spp. exhibit strong dormancy and only a small number of them will germinate in the following season. Dormancy in this genus is linked to several factors, especially physical attributes that are related to seed coat restrictions imposed by the glumes (lemma and palea), pericarp and tegument (Binotti et al., 2014). Understanding the mechanisms of dormancy and its duration for various species, as well as collaborating in defining the need or not to use specific treatments to interfere in the metabolism of the seed, releasing the embryo to development or making it suitable for germination, has increased the ecological and economic importance (Dias, 2005). However, dormancy can be overcome with treatments performed in the laboratory, such as mechanical scarification (sanding), chemical (acids) and thermal (hot water). These methods cause the seed coat to be disrupted, allowing water to enter and stimulating metabolic processes (Borges &

Rena, 1993). Under laboratory conditions, seed technologists recommend chemical scarification using sulfuric acid to overcome dormancy. In addition, supplying the germination substratum with potassium nitrate (KNO_3) and oven-dry thermal treatment can be used for dormancy release (Batista et al., 2016). The germination of seeds of the genus *Urochloa* has been investigated by several authors (Grof, 1968; González et al., 1994; Voll et al., 1997). Grof (1968); Renard & Capelle (1976) observed that seed covers, glume, palea, and lemma, constituted a barrier to germination due to impermeability to water and oxygen. Besides the dormancy caused by seed covers, an endogenous dormancy is supposed to block the germination of freshly harvested seeds. This endogenous or physiological dormancy could be associated with the balance of growth regulators in the seed (Khan, 1971; Bewley & Black, 1994). In *U. brizantha* cv. Marandu, Garcia & Cícero (1992) observed that the treatment of the seeds with sulfuric acid promoted 15 to 18% germination, while gibberellic acid caused 14% germination. *Urochloa* species normally show a double seed dormancy mechanism, mainly on fresh-harvested seeds, leading to germination percentages lower than those of viability detected by tetrazolium test (TZ) and causing problems in storage, trading, and seed inspection activities (Filho & Usberti, 2008). A fast germination is highly desirable for successfully establishing forage grass pasture fields. However, seed dormancy could contribute for the setting and the persistence of the stand and avoiding the occurrence of the germination immediately after the harvest (Herrera, 1994).

According to Whiteman & Mendra (1982), dormancy in *Urochloa* seeds is controlled by two mechanisms, the physiological dormancy presents in freshly harvested seeds and the mechanical dormancy imposed by the wrappings, related to the restrictions of the entry of oxygen and water in the seeds. The physiological dormancy associated with the embryo is progressively suppressed during storage and the mechanical dormancy imposed to the embryo persists in seeds storage for long periods. Despite the evident importance of dormancy in freshly harvested seeds, the mechanisms that are involved in overcoming dormancy that occurs during storage are still poorly elucidated. Because of this, some methods are recommended for the total overcoming of seed dormancy, such as chemical treatments, mechanical scarification, and heat treatments with the use of elevated temperatures. For seeds of *Urochloa*, chemical scarification with sulfuric acid has been recommended (de Souza e Silva et al., 2014). Dormancy expression in tropical grasses is associated with physiological causes (present in freshly harvested seeds and suppressed during storage) or physical causes (related to restrictions imposed by the seed coat to oxygen and water uptake by seeds) (Whiteman & Mendra, 1982).

The dormancy in one of the mixtures of grass species in the pastureland can lead to complete elimination of the species during the establishment phase. Hence, breaking the dormancy of freshly harvested seeds is necessary for improving the germination. The seed may require different types of treatment to break seed dormancy and to make the seed readily germinative in the forthcoming seasons. Seed dormancy breaking treatment can be given to the seed based on the type and place of seed dormancy (Shanmugavalli et al., 2007).

2. Problem statement and justification

The demand for high quality forage seed for development of livestock feed resources is increasing rapidly across the globe. This demand is being fueled by increased beef and dairy production as a result of rising populations and incomes. The availability of adequate, good quality forage seed is critical to meeting the expanding meat and milk demand in the world. Furthermore, the need to restore degraded natural pasture (the major source of livestock feed) through reseeding and/or over-sowing interventions emphasizes the urgency for concerted efforts to ensure the availability of large quantities of good quality seeds (Kabirizi, n.d.). However, production of adequate quantities of good quality seed is greatly constrained by inadequate knowledge of appropriate agronomic practices among seed growers, especially in new cultivars adopted. Research studies to obtain information on appropriate forage seed production techniques for farmers are thus critical in ensuring the availability of adequate quantities of good quality seed. Consequently, new forage species require the development of specific technology to meet their seed production needs, and the continuing commercialization of new species means a continuing need for support research on seed production (Schultze-Kraft, 1995; Hacker & Loch, 1998).

In Thailand, pasture grass research in the past emphasized on the introductions, evaluations, and selection of the grasses in government station evaluation trials conducted by the Bureau of Animal Nutrition Development, Department of Livestock Development (DLD) in different agro-ecological locations. The main evaluation criteria used in these investigations included persistence, dry matter production, resistance to grazing, drought tolerance, and quality, mainly the chemical composition, in vivo and in vitro digestibility. Additional trials were also carried out to evaluate the responses of promising grass species/cultivars to various agronomic management practices. There is limited information available on the seed production of promising grass species and cultivars in various agro-ecological locations, as well as their production under various agronomic management practices.

A few years ago, two novel cultivars of *Urochloa* spp. were introduced to Thailand after genetic trait pre-selection in Japan. The candidate cultivar 'Br-203' (*Urochloa ruziziensis* (R. Germ. & C.M. Evrard) Crins × *Urochloa brizantha* (Hochst. Ex A. Rich.) Stapf) is an apomictic hybrid produced by crossing a sexual maternal tetraploid parent (i.e., 'Miyaokikoku' (*Urochloa ruziziensis* cv. Miyaokikoku-ichigou) (Ishigaki et al., 2009a)) with an apomictic paternal tetraploid parent (i.e., 'Mulato' (*Urochloa ruziziensis* × *Urochloa brizantha*)). A new candidate, cv. 'Br-203', was selected using MAS for apomixis and selected breeding criteria. After pre-selection by MAS for apomixis trait in Japan, effective and compact breeding populations have been transferred to the DLD Thailand. It exhibits apomixis and produces a high forage yield and good forage quality with wide, long leaves, which indicates comparatively low maturity seed and moderately frequent flowering at the moderate flowering time (Nakmanee et al., 2015). Major agronomical traits selections have been successfully performed in Thailand. The other candidate cultivar, 'OKI-1' is an open-pollinated cultivar (Kouki et al., 2014) that was developed by recurrent selection through the evaluation of seed fertility and plant productivity among the base population of tetraploid *Urochloa ruziziensis* 'Miyaokikoku' (Ishigaki et al., 2009a). The cultivar 'OKI-1' was also obtained by the DLD Thailand after pre-selection of the desired traits in Japan. The breeding scheme of both new cultivars is shown in Figure 1-1.

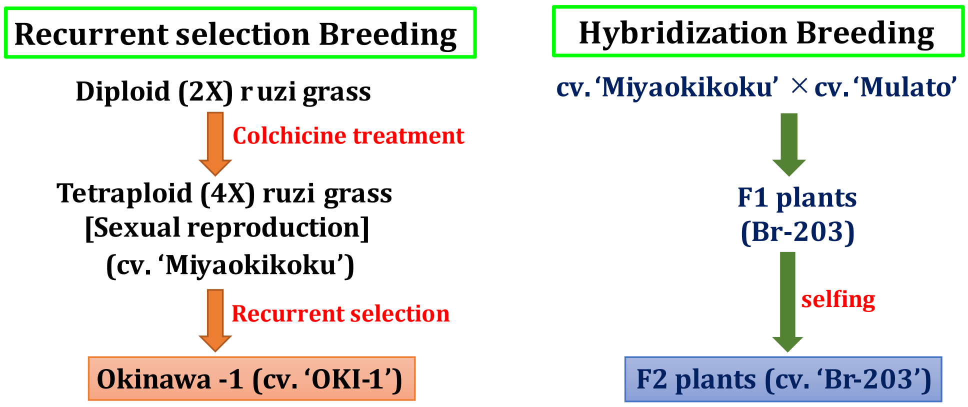

Figure 1-1 The breeding scheme of two new *Urochloa* spp., cv. 'OKI-1' and cv. 'Br-203'.

The cultivar 'Br-203' exhibited a high forage dry matter yield (19,200 kg/ha/year over an average of two consecutive years at five test sites in Thailand), good palatability, good regrowth and extension, moderately frequent flowering, and a seed maturity rate that was similar to that of 'Basilisk' (Nakmanee et al., 2015). In a preliminary trial at the Lampang Animal Nutrition Research and Development Center (ANRDC) in 2017, cv. 'OKI-1' had a dry matter yield of 15,800 kg/ha/year. In addition, cv. 'OKI-1' had significantly higher *in vitro* dry matter digestibility (IVDMD) at all stages of growth and in all parts of the plant compared to

U. ruziziensis, Mulato II (*U. ruziziensis* × *U. decumbens* (Stapf) R.D. Webster × *U. brizantha* (Hochst. ex A. Rich.) R.D. Webster) and *U. decumbens* cv. Basilisk (Thaikua et al., 2018).

Production of quality grass seeds needs special attention in establishment, field crop management, seed harvesting, and processing. There is little information available on the seed production of these new grass cultivars in Thailand, their response to different closing cut dates, N fertilizer application rates, plant spacing, and harvesting method under field conditions. Consequently, there was a need to evaluate these agronomic practices on how they affect seed yield and quality of both cultivars in the climate of the Northern Thailand and hence establish seed production field criteria based on study components for improvement.

3. Objectives

The main objective of this Ph.D. dissertation was to evaluate the performance in seed yield and seed quality of two novel cultivars of *Urochloa* spp., cv. 'Br-203' and cv. 'OKI-1' under various agronomic practices in Northern Thailand. In addition, the best method for overcoming seed dormancy in the promising candidate cultivar that can be produced for commercialization was evaluated. The results of this study are expected to be used as the basic information applied to cultivating the promising candidate cultivars in other countries and areas where environmental conditions are similar to those in the northern part of Thailand. The main objective of this study has been addressed through the following specific objectives:

(1) Evaluate seed yield and seed quality of cv. 'Br-203' and cv. 'OKI-1' compared with *Urochloa* hybrid cv. 'Mulato II', *U. decumbens* cv. 'Basilisk', and *U. ruziziensis* cv. 'Kennedy'.

(2) Evaluate the appropriate agronomic management practices, i.e., plant spacing, closing cut date, applying nitrogen fertilizer, and seed harvesting method for maximizing seed yield and seed quality in cv. ‘Br-203’ and cv. ‘OKI-1’.

(3) Evaluate the seed imbibition and different treatments for breaking seed dormancy of the promising candidate *Urochloa* spp. (cv. ‘OKI-1’).

CHAPTER 2

A comparative study on seed yield and seed quality of two novel cultivars and commercial cultivars of *Urochloa* spp.

Abstract

Two novel cultivars (cv.) of *Urochloa* spp. (synonym *Brachiaria* spp.), cv. 'OKI-1' (an open-pollinated tetraploid *Urochloa ruziziensis* (R. Germ. and C.M. Evrard) Crins originated from cv. 'Miyaokikoku-ichigou') and cv. 'Br-203' (*U. ruziziensis* cv. 'Miyaokikoku-ichigou' × *U.* hybrid cv. 'Mulato') were evaluated for seed yield, seed yield components, and seed quality in 2018–2020 at the Lampang ANRDC, Northern Thailand. These cultivars were compared with *U. decumbens* cv. 'Basilisk', *U. ruziziensis* cv. 'Kennedy', and *Urochloa* hybrid, cv. 'Mulato II' (*U. ruziziensis* × *U. decumbens* (Stapf) R.D. Webster × *U. brizantha*). Comparisons of the five grass cultivars were made in a randomized complete block design with three replicates. The results showed the variation in seed yield and its components, as well as seed quality. On a two-year average, the pure germinated seed yield (PGSY) obtained from cv. 'Kennedy', cv. 'Mulato II', cv. 'OKI-1', cv. 'Br-203', and cv. 'Basilisk' was 359.5, 336.5, 320.6, 137.9, and 115.2 kg/ha, respectively. Although the PGSY between the cultivars had no significant difference, the pure seed yield (PSY) of cv. 'Mulato II' and cv. 'Kennedy' was significantly higher than cv. 'Br-203' and cv. 'Basilisk' ($p < 0.05$). However, the PSY of cv. 'OKI-1' was not different from that of cv. 'Mulato II' and cv. 'Kennedy' ($p > 0.05$). When seed yield and seed quality were considered, cv. 'OKI-1' demonstrated seed production potential comparable to the other commercial cultivars, namely cv. 'Kennedy' and cv. 'Mulato II'. Consequently, the planting of cv. 'OKI-1' for commercial seed production could be viable.

Introduction

The quantity and quality of forage produced are the primary selection criteria in forage grass breeding, but efficiency in seed production is also important during the seed multiplication phase of breeding a new cultivar. Seed yield is a trait of major interest in forage grass species as seed multiplication is economically important for new grass cultivars to become commercially viable (Boelt & Studer, 2010). Seed quality, which includes genetic and physical purity, viability, vigor, and seed health, all of which are important in a competitive market.

Thailand's livestock population has grown rapidly in the last decade as a direct result of government promotion (Wattanachant, 2019). As a result, there is a greater demand for pasture. A 6-month dry season, poor soils, and a scarcity of drought-tolerant species all limit pasture productivity. Ruzigrass (*U. ruziziensis*) has been promoted and widely grown, owing to its ease of seed production (Hare et al., 2005). Signalgrass (*U. decumbens*) has long been recognized as being a pasture species suitable for planting in Northeast Thailand. It is drought-tolerant and outperforms ruzigrass in terms of forage production in the dry season. Its use in Thailand has been limited due to extremely low seed yields. The *Urochloa* hybrid, cv. 'Mulato II' (*U. ruziziensis* × *U. decumbens* (Stapf) R.D. Webster × *U. brizantha*) was introduced into Thailand in 2003. It has high forage quality, drought tolerance, and persistence, which results in demand for its seed having rapidly increased. However, in Thailand, cv. 'Mulato II' seed yields have always been less than half of ruzigrass seed yields (Hare, 2021). A few years ago, two novel cultivars of *Urochloa* spp., i.e., cv. 'Br-203' and cv. 'OKI-1', were introduced into Thailand after pre-selection of desired traits in Japan. They have exhibited high potential as pasture crops in both terms of forage yield and quality when grown in Thailand. Until now, research on seed production of both cultivars has been limited. Thus, the objective of the study was to evaluate the seed production performance of the two new cultivars compared with the three commercial cultivars used in Thailand.

Materials and Methods

Experimental sites

The field trial was conducted at the Lampang ANRDC, Hang Chat, Lampang, Northern Thailand (18.3°N, 99°E; 320 m asl) from May 2018 to February 2020. The soil is classified as the Renu soil series, which is characterized by sandy loam to sandy clay loam soil, poor drainage, and low fertility (Land Development Department (LDD), 2020) as shown in Table 2-1.

Meteorological Data

In 2018 and 2019, data on temperature, precipitation, rainy days, sunshine duration, relative humidity, and day length at the Lampang ANRDC were collected from the Hang Chat Agrometeorological Station in Hang Chat, Lampang, Thailand, which is about 2 km away from the experimental sites. The meteorological data, i.e., the annual rainfall and rainy days, air temperatures and humidity, and sunshine duration and day length during the study period are shown in Figure 2-1 (A), (B), and (C), respectively.

Plant materials

(1) cv. ‘Br-203’, a candidate cultivar of apomictic *Urochloa* hybrid (tetraploid *U. ruziziensis* × *Urochloa* hybrid cv. ‘Mulato’)

(2) cv. ‘OKI-1’, a candidate cultivar of open-pollinated tetraploid *U. ruziziensis*

(3) cv. ‘Mulato II’, a commercial cultivar of apomictic *Urochloa* hybrid (tetraploid *U. ruziziensis* × *U. decumbens* × *U. brizantha*)

(4) *U. decumbens* cv. Basilisk, a commercial cultivar of apomictic tetraploid *U. decumbens*

(5) *U. ruziziensis* cv. Kennedy, a commercial cultivar of open-pollinated diploid *U. ruziziensis*

Experimental design

Two to three tillers with roots from a one-year-old plant were used to clonally propagate each cultivar. Each one had been grown in a culture bag containing soil for one month, then they were transplanted into an experimental field using a randomized complete block design (RCBD) with three replications. The plot size was 4 × 5 m and comprised of twenty plants with a spacing of 1 × 1 m.

Data collection and seed harvesting

All seedheads within each plot, except for the border row, were harvested over the period of seed maturity (late November–early December). For only cv. 'Basilisk', which is day-neutral in photoperiod response, the first flowering emergence started in mid-August, resulting in its matured seeds being collected earlier than the other cultivars. The total number of tillers per plant (TN/P) was counted on three randomly selected plants in each plot at the time of harvest. The number of inflorescences per tiller (IN/T) was counted from 15 randomly selected tillers from the three selected plants. The number of racemes per inflorescence (RN/I) was counted from 15 randomly selected inflorescences of the three selected plants. The number of spikelets per raceme (SN/R) was averaged from the upper, mid, and lower racemes of each inflorescence, which was randomly selected from the 15 inflorescences of the same three selected plants. Tiller fertility was expressed as the percentage of inflorescences over the total number of tillers. All seedheads in each plot were tied together into manageable bunches when the seed was almost ripe. Nylon net bags were used to cover the bunch, and each bag was tied to a bamboo stake; the bag remained in place for the duration of the harvest. Bags were used to collect all the seeds produced. Ripe seeds were threshed off the inflorescences by lightly tapping the nylon net bag. The plots were harvested individually.

Seed processing and calculation of secondary attributes

The seed was air-dried for several days in the shade, before being sun-dried for a few days and then cleaned using a hand screen and seed blower. Seed yield (SY) was measured for each plot. Seed moisture content (SMC), thousand seed weight (TSW), purity percentage (PP) and germination percentage (GP) were determined following the test methods of the International Seed Testing Association for *Urochloa ruziziensis* (ISTA, 2011). TSW was determined from pure-seed spikelet weight. Purity analysis was performed by separating the pure seed fraction from inert matter and other seeds in each sample, and then separately weighing the pure seed fraction. The PP was calculated as the (weight of pure seed) × 100/(total weight of sample). The germination test was performed at 90 days after seed harvesting in each experimental year. SY was corrected to 11% SMC. The fertile tiller percentage (FTP), tiller number/m^2 (TN), inflorescence number/m^2 (IN), number of fertile tillers/m^2 (FTN), and filled seed percentage (FSP) were calculated. The FTP was calculated as FTN × 100/TN, pure seed yield (PSY) was calculated as SY × PP/100, and the pure germinated seed yield (PGSY) was calculated as PSY × GP/100.

Statistical analysis

Data on seed yields, seed yield components, and seed quality attributes of five *Urochloa* spp. were subjected to analysis of variance (ANOVA) based on the model designed for a randomized complete block design (RCBD), and the least significant difference (LSD) was carried out for subsequent comparison of means between cultivars by using R (R Core Team, 2020).

Table 2-1 Chemical properties of initial soil in the experimental field

pH	OM (%)	Total N (%)	Extractable (mg/kg)										EC (dS/m)
			P	K	Ca	Mg	Na	S	Mn	Fe	Cu	Zn	
5.28	0.62	0.03	0.69	48.50	303.08	29.90	9.43	2.92	12.24	88.79	0.16	0.05	0.62

Abbreviations: EC, electric conductivity; OM, organic matter.

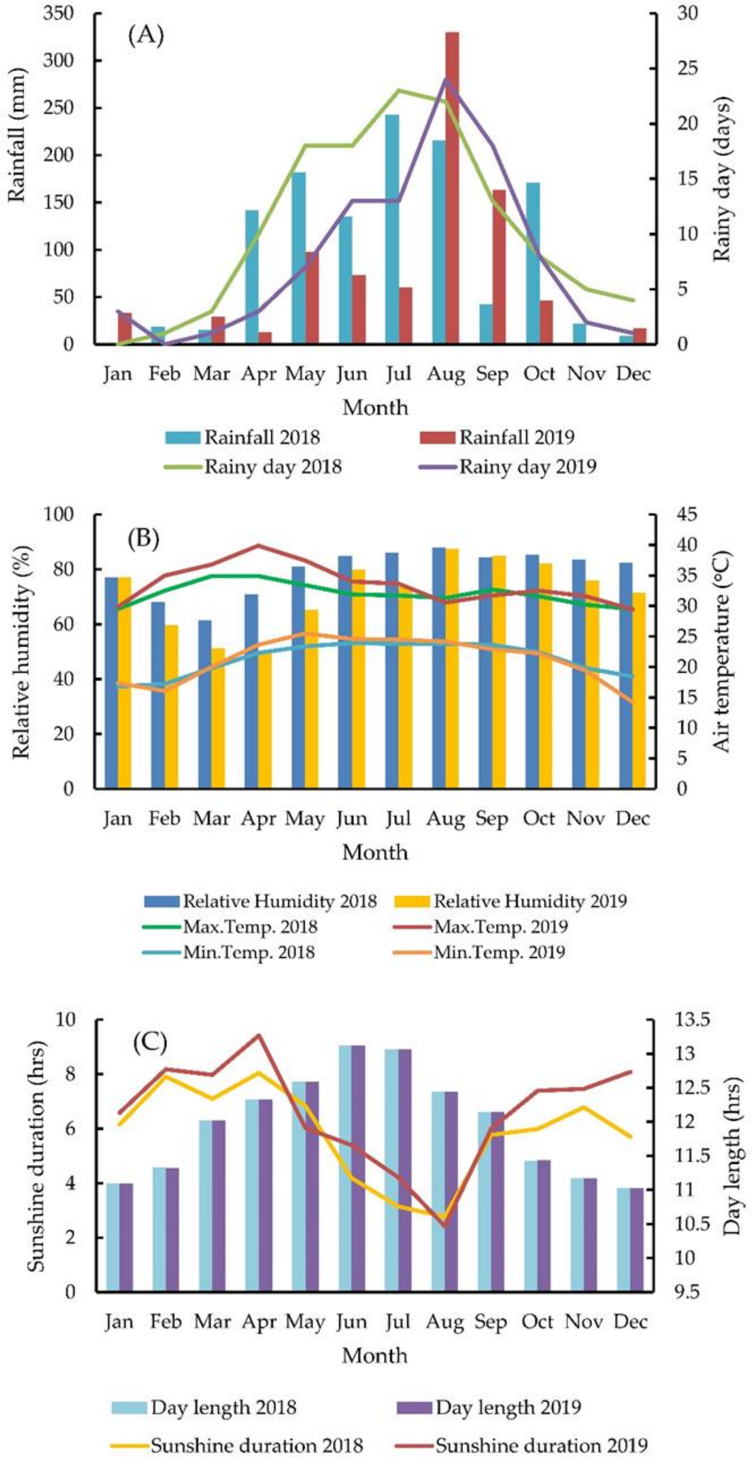

Figure 2-1 Meteorological data at the Lampang Animal Nutrition Research and Development Center in 2018 and 2019: (**A**) The average monthly rainfall and the number of rainy days in a month; (**B**) The monthly average relative humidity, the monthly average minimum, and the maximum temperature; (**C**) The monthly average day length and average daily sunshine duration.

Results

Seed yield and its components, and seed quality of the study are shown in Tables 2-2 and 2-3, respectively. Over a two-year average, the PGSY between cultivars was not significantly different ($p > 0.05$), though a significant difference between cultivars was observed in each year of the experiment. In 2018, the highest PGSY was obtained from cv. 'Mulato II' (528.39 kg/ha), and cv. 'Basilisk' showed the lowest (118.16 kg/ha). In 2019, the highest PGSY was obtained from cv. 'OKI-1' (395.75 kg/ha), and cv. 'Br-203' showed the lowest (44.93 kg/ha). Even though significant differences between cultivars were observed for the TSY and PSY, the PGSY did not show a statistically significant difference. The highest TSY was obtained from cv. 'Mulato II' (681.08 kg/ha), and it differed significantly from the other cultivars. The highest PSY was also obtained from cv. 'Mulato II' (621.47 kg/ha), followed by cv. 'Kennedy' (454.92 kg/ha), cv. 'OKI-1' (377.98 kg/ha), cv. 'Br-203' (189.41 kg/ha) and cv. 'Basilisk' (182.79 kg/ha).

Based on seed yield components in a two-year average, all measured traits had significant differences between cultivars. The cv. 'Kennedy' had the highest TN and FTN, but the cv. 'Br-203' showed the lowest. The lowest FTP and SN/R were obtained from cv. 'Basilisk', and they differed significantly from the other cultivars. The highest IN was obtained from cv. 'Mulato II', followed by cv. 'Br-203', cv. 'OKI-1', cv. 'Kennedy', and cv. 'Basilisk'. The cv. 'Br-203' and cv. 'Mulato II' had a significantly higher IN/T than the other cultivars, while those two cultivars, as well as cv. 'Basilisk', had the lowest RN/I. Most seed quality attributes, except for PP, showed a significant difference between cultivars in a two-year average. The FSP was highest in cv. 'Kennedy', while the lowest in cv. 'Basilisk' and cv. 'Br-203'. The TSW of cv. 'OKI-1' was significantly higher when compared with cv. 'Basilisk' and cv. 'Kennedy'. Furthermore, when compared with cv. 'Mulato II', cv. 'OKI-1' had a significantly higher GP.

Table 2-2 Seed yield and seed yield components in the five *Urochloa* cultivars

Year	Cultivar	TSY (kg/ha)	TN (no./m^2)	FTN (no./m^2)	IN/P (no./ m^2)	FTP (%)	IN/T (no.)	RN/I (no.)	SN/R (no.)
2018	Basilisk	186.93^{c}	103.17	64.94^{b}	152.40^{b}	62.78^{b}	2.72^{bc}	3.18^{c}	26.26^{bc}
	Mulato II	899.41^{a}	114.17	90.33^{a}	374.33^{a}	80.22^{a}	4.33^{ab}	3.30^{c}	32.68^{a}
	Kennedy	534.12^{b}	122.44	97.61^{a}	193.47^{b}	80.66^{a}	2.28^{bc}	4.87^{a}	25.81^{c}
	OKI-1	306.03^{bc}	113.72	86.89^{a}	176.21^{b}	76.05^{a}	1.50^{c}	4.09^{b}	30.57^{a}
	Br-203	314.83^{bc}	102.78	80.89^{ab}	408.68^{a}	78.79^{a}	5.28^{a}	3.27^{c}	28.15^{b}
2019	Basilisk	264.00^{b}	272.17^{a}	206.61^{ab}	410.76^{b}	75.81^{c}	2.02^{bc}	2.84^{bc}	25.58^{b}
	Mulato II	462.75^{a}	254.83^{a}	216.50^{ab}	620.38^{a}	84.81^{ab}	2.87^{a}	2.56^{c}	33.58^{a}
	Kennedy	319.47^{ab}	280.89^{a}	234.78^{a}	391.19^{b}	84.05^{b}	1.67^{c}	3.16^{ab}	36.27^{a}
	OKI-1	398.58^{ab}	208.33^{b}	190.83^{bc}	479.99^{ab}	91.30^{a}	2.41^{ab}	3.51^{a}	35.72^{a}
	Br-203	235.26^{b}	193.67^{b}	156.89^{c}	442.11^{ab}	80.55^{bc}	2.78^{a}	2.72^{c}	36.13^{a}
2-year average	Basilisk	225.46^{b}	187.67^{ab}	135.78^{bc}	281.58^{c}	69.29^{b}	2.37^{b}	3.01^{b}	25.92^{b}
	Mulato II	681.08^{a}	184.50^{ab}	153.42^{ab}	497.36^{a}	82.51^{a}	3.60^{a}	2.93^{b}	33.13^{a}
	Kennedy	426.80^{b}	201.67^{a}	166.19^{a}	292.33^{c}	82.36^{a}	1.98^{b}	4.01^{a}	31.04^{a}
	OKI-1	352.31^{b}	161.03^{bc}	138.86^{bc}	328.10^{bc}	83.67^{a}	1.96^{b}	3.80^{a}	33.15^{a}
	Br-203	275.05^{b}	148.22^{c}	118.89^{c}	425.39^{ab}	79.67^{a}	4.03^{a}	2.99^{b}	32.14^{a}

Abbreviations: FTN, fertile tiller number per square meter; FTP, fertile tiller percentage; IN, inflorescence number per square meter; IN/P, inflorescence number per plant; IN/T, inflorescence number per tiller; RN/I, raceme number per inflorescence; SN/R, spikelet number per raceme; TN, tiller number per square meter; TSY, total seed yield. Note: Values with different superscript letters indicate statistical differences within each column for each year or 2-year average (p < 0.05).

Table 2-3 Seed quality in the five *Urochloa* cultivars

Year	Cultivar	FSP (%)	TSW (g)	PP (%)	PSY (kg/ha)	GP (%)	PGSY (kg/ha)
2018	Basilisk	10.68^{c}	6.05^{c}	98.10	183.57^{c}	65.33	118.16^{b}
	Mulato II	19.88^{bc}	8.20^{ab}	97.80	879.24^{a}	59.67	528.39^{a}
	Kennedy	40.00^{a}	6.37^{bc}	98.40	526.03^{b}	74.00	394.82^{ab}
	OKI-1	29.64^{ab}	9.91^{a}	99.67	305.00^{bc}	80.67	245.43^{ab}
	Br-203	10.72^{c}	7.85^{bc}	99.20	312.44^{bc}	74.33	230.90^{ab}
2019	Basilisk	14.46^{b}	6.22^{b}	93.53	182.00^{bc}	63.67^{ab}	112.17^{bc}
	Mulato II	21.02^{ab}	7.55^{ab}	93.00	363.70^{ab}	43.67^{b}	144.61^{abc}
	Kennedy	40.54^{a}	5.79^{b}	95.33	383.82^{ab}	82.33^{a}	324.19^{ab}
	OKI-1	34.92^{a}	9.09^{a}	97.63	450.96^{a}	86.00^{a}	395.75^{a}
	Br-203	5.67^{b}	6.95^{ab}	93.17	66.38^{c}	60.67^{ab}	44.93^{c}
2-year average	Basilisk	12.57^{c}	6.14^{b}	95.82	182.79^{b}	64.50^{ab}	115.16
	Mulato II	20.45^{bc}	7.88^{ab}	95.40	621.47^{a}	51.67^{b}	336.50
	Kennedy	40.27^{a}	6.08^{b}	96.87	454.92^{a}	78.17^{ab}	359.50
	OKI-1	32.28^{ab}	9.50^{a}	98.65	377.98^{ab}	83.33^{a}	320.59
	Br-203	8.19^{c}	7.40^{ab}	96.18	189.41^{b}	67.50^{ab}	137.91

Abbreviations: FSP, filled seed percentage; GP, germination percentage; PGSY, pure germinated seed yield; PP, purity percentage; PSY, pure seed yield; TSW, thousand seed weight. Note: Values with different superscript letters indicate statistical differences within each column for each year or 2-year average ($p < 0.05$).

Discussion

One of the areas of interest in the study was inflorescence density as an indicator of whether species could flower abundantly, which would overcome the first barrier for seed production. Both cv. 'OKI-1' and cv. 'Br-203' produced sufficient inflorescences, racemes, and spikelets to indicate a potential for useful seed yields. However, cv. 'Br-203', had a massive failure of seed-set, caryopsis maturation, or both, with the cleaned seed containing less than 10% of the spikelets formed by the crops. The variation in all seed yield component traits of each cultivar in a two-year trial was discovered. These results showed that the highest TN and FTN were obtained from *U. ruziziensis* cv. 'Kennedy'. For cv. 'Br-203' and cv. 'OKI-1', which were developed from *U. ruziziensis*, the TN and FTN were significantly lower than cv. 'Kennedy'. On the other hand, cv. 'Kennedy' had lower IN and IN/T than cv. 'Br-203' but was similar to cv. 'OKI-1'.

Although significant differences in TSY and PSY between cultivars were found, PGSY had no significant difference. This result was caused by the difference in GP value in each cultivar. For example, the cv. 'Mulato II' had the highest TSY and PSY (681.08 kg/ha and 621.47 kg/ha, respectively) but the lowest GP (51.67 percent), resulting in a PGSY (336.50 kg/ha) value that was comparable to the other cultivars. In the second year of the experiment (2019), most cultivars produced lower PSY and PGSY compared with the first year, except for cv. 'OKI-1', which increased by about 32% and 38%, respectively. The results coincide with Nakmanee et al. (2007a) who reported that PSY of cv. 'Kennedy' and cv. 'Mulato', which were harvested in Northeast Thailand, decreased in the second-year crops by 34% and 37%, respectively. The study did not explain why the decrease occurred. Furthermore, when compared to the other the two cultivars, cv. 'Mulato II' had the significantly lower PSY. They explained that the emergence of inflorescences of cv. 'Mulato II' was too late. The flowering period occurred in a dry season (October to November), in which the low humidity affected seed setting. This was confirmed by Loch (1980), who stated that reduced seed set has been associated with low humidity during anthesis.

The cv. ‘OKI-1’ exhibited the highest PGSY in the second year of the experiment, especially higher than the cv. ‘Br-203’ and cv. ‘Basilisk’. Even though plant utilization reduced soil nutrients from the first year to the second year of the experiment, cv. ‘OKI-1’ was still able to produce more seed under this stress. Besides the traits of the species that affect seed yields, its adaptability to environmental stresses is also important. The contrasting environmental conditions that occur during the vegetative and reproductive growth of the crops may result in seed yield instability (Clua & Gimenez, 2003). Generally, photoperiod (day length) and temperature are the primary environmental factors that control flowering in tropical forage grasses, and they interact with rainfall and humidity to determine the suitability of different locations for seed production (Hopkinson et al., 1996). Furthermore, drought stress and dehydration (low humidity), nitrogen supply, and other factors can reduce pollen viability. (Chastain, 2013). Drought during seed development usually disrupts seed development and results in light, shriveled seed. In 2019, insufficient rainfall during vegetative growth, high temperatures, and lower humidity during seed setting may lead to a low PGSY in most of the tested grasses. The weather conditions in a two-year experiment are shown in Figure 2-1. In terms of commercial seed production, this area site is suitable for cv. ‘OKI-1’ as well as cv. ‘Mulato II’ and cv. ‘Kennedy’.

In this study, cv. ‘Mulato II’ produced higher TSY than the other cultivars, and its PSY was more than 600 kg/ha. In 2018, the significantly highest TSY was obtained from cv. ‘Mulato II’ (899.41 kg/ha), but in 2019, the TSY was about 49% decreased. However, there was more than the seed yield of cv. ‘Mulato II’, which was produced in Northeast Thailand by Hare et al. (2015) and Nakmanee (2007a), who reported that the seed yield was 232–258 kg/ha, and 106–131 kg/ha, respectively. Hare et al. (2015) recommended that to compete in price internationally with commercial *Urochloa* cultivars from Brazil and Australia, commercial seed yields from hybrid *Urochloa* must be at least 600–700 kg/ha. Therefore, cv. 'Mulato II' seed production in Northern Thailand showed the potential in the competition. They also stated that the seed yield of cv. 'Mulato II' produced in Northeast Thailand was still very low compared with yields from other commercial *Urochloa* cultivars (not hybrids) elsewhere. Commercial seed yields averaged 650–700 kg/ha in Brazil for cv. 'Marandu' (*U. brizantha*) and cv. 'Basilisk' (*U. decumbens*) (de Souza, 1999). In Australia, seed yields of cv. 'Basilisk' have reached 1,000 kg/ha (Hopkinson & Clifford, 1993). In contrast with the present study, the PSY of cv. 'Basilisk' was the lowest, at less than 200 kg/ha, similar to that of cv. 'Br-203'. The low seed yield of cv. 'Br-203' may be caused by the disadvantages of cv. 'Mulato', which is its apomixis paternal tetraploid parent (i.e., 'Mulato' (*U. ruziziensis* × *U. brizantha*)). This is supported by the study of Hare et al. (2007b), who reported that the seed yield of cv. 'Mulato' (161 kg/ha) was 60% lower than the seed yield of cv. 'Mulato II' (258 kg/ha). Moreover, cv. 'Br-203' produced plenty of empty seeds, which is indicated by the very low value of FSP. As a result, low seed yields were obtained. Therefore, seed production of cv. 'Br-203' is not successful for endorsement in the commercial seed market, just like the cv. 'Basilisk', which has not been successfully used for seed production in Thailand. Although cv. 'Basilisk' is drought-tolerant and carries its production in the dry season much better than cv. 'Kennedy', its use in Thailand has been limited by very poor seed yields (Gobius et al., 2001). Because cv. 'Kennedy' produces a high seed yield with high FSP and GP, the cultivar is still popular for seed production in Thailand. This can be confirmed by the results of the present study. Despite the fact that cv. 'OKI-1' showed a value of FSP similar to that of cv. 'Kennedy' as well as TSW was higher than that of cv. 'Kennedy', its PSY was rather lower than that of cv. 'Kennedy'. This is caused by the lower TSY. However, cv. 'OKI-1' seed production could be improved by agricultural management practices because, at present, the knowledge about its growth and reproductive behaviors as well as the proper management that is suited for seed production of this cultivar is limited.

Conclusion

The main outcome of the seed production among two novel cultivars of *Urochloa* spp. from this study was that only the seed yield of cv. ‘OKI-1’ was commercially viable and comparable to the seed yields of cv. ‘Kennedy’ and cv. ‘Mulato II’. The cv. ‘Br-203’ seed yields appeared to be high, but they had a lot of empty seeds, so the weight of TSY, PSY, and PGSY was very low. Further research should be conducted in different locations with varying climatic conditions, soil types, and economic feasibility to investigate appropriate agronomic and management practices to maximize seed production and productivity of grass species in different study areas because each grass species has different agronomic requirements.

Effects of plant spacing on seed yield and seed quality in new *Urochloa* cultivars

Abstract

Urochloa (syn. *Brachiaria*) cultivars are widely used as forage for ruminants in tropical countries, including Thailand. Two new *Urochloa* cultivars, ‘OKI-1’ and ‘Br-203’, have high forage yield and digestibility compared with other cultivated *Urochloa* grasses. Seed production is a fundamental requirement for widespread cultivation, and it can be increased by optimizing agronomic practices, such as changing plant spacing. Two field trials were conducted in 2018–2020 at the Lampang ANRDC, Northern Thailand to determine the optimal plant spacing for seed production of these *Urochloa* cultivars. In both trials, four plant-spacing regimes (50 × 100 cm, 75 × 100 cm, 100 × 100 cm, and 125 × 100 cm) were used in RCBD with four replicates. In two consecutive harvests of both trials, plant spacing did not significantly affect seed yield and quality but affected tiller and inflorescence densities. Specifically, significant higher numbers of tillers and inflorescences were produced per unit area at the narrowest plant spacing (i.e., 50 × 100 cm). The cultivars ‘OKI-1’ and ‘Br-203’ tended to have higher PSY at plant spacings of 100 × 100 cm (136.46 kg/ha) and 75 × 100 cm (79.59 kg/ha), respectively. Both cultivars showed similar trends in PSY, FSP and TSW, which tended to be higher in the first-year crops than the second-year crops. This difference could be attributed to a reduction in available soil N resulting from a large amount of N utilization during vegetative growth, combined with inadequate and erratic rainfall. In addition to the recommended optimum plant spacing, sufficient fertilizer and suitable environmental factors could increase seed yield.

Introduction

The genus *Urochloa* (synonym *Brachiaria*) contains approximately 100 species, including several commercially important species, and is probably the most widely distributed genus used for forage grass in the tropics (Fisher & Kerridge, 1996). In addition to their high nutritive value, their adaptability and environmental benefits (e.g., reducing greenhouse gas emissions and soil erosion) are also remarkable. *Urochloa* spp. are used by ruminant livestock producers due to their high forage yield and palatability for cattle (Rao et al., 2015). *Urochloa* spp. can be used in pasture, used as forage under cut-and-carry systems, hay, silage, and mixed grass-legumes. Grasses in genus *Urochloa* have been accepted to alleviate livestock feed shortages, increase the availability and quality of feeds, improve livestock productivity and farmers' sources of income (Cheruiyot et al., 2020).

The success of new forage species depends on their palatability, ability to produce dry matter, nutritional quality, yield stability, pest and disease resistance, and high capacity to produce viable seeds (Lopes et al., 2017). A new candidate cultivar, 'Br-203' (*Urochloa ruziziensis* (R. Germ. & C.M. Evrard) Crins × *Urochloa brizantha* (Hochst. Ex A. Rich.) Stapf), which is an apomictic hybrid produced by crossing a sexual maternal tetraploid parent (i.e., 'Miyaokikoku' (*Urochloa ruziziensis* cv. Miyaokikoku-ichigou) (Ishigaki et al., 2009a)) with an apomixis paternal tetraploid parent (i.e., 'Mulato' (*Urochloa ruziziensis* × *Urochloa brizantha*)), In addition, another candidate cultivar, 'OKI-1', which is an open-pollinated cultivar (Kouki et al., 2014) developed by recurrent selection of a base population of the tetraploid *Urochloa ruziziensis* 'Miyaokikoku' (Ishigaki et al., 2009a), was obtained by the DLD, Thailand after pre-selection of desired traits in Japan. Major selection of agronomic traits of both cultivars was successfully performed in Thailand in 2017. Specifically, cv. 'Br-203' exhibited apomixis, high forage yield (19.2 t/ha/year over an average of two consecutive years at five test sites in Thailand), good palatability with a wide and long leaf, good regrowth and extension, moderately frequent flowering, and seed maturity rate was similar to cv. 'Basilisk' (Nakmanee et al., 2015). The other field trial conducted by the Thailand DLD from October 2016 to October 2017 in Lampang, showed that cv. 'OKI-1' produced a dry matter yield of 15.8 t/ha/year with significantly higher *in vitro* dry matter digestibility (IVDMD) at all stages of growth and in all parts of the plant compared to *U. ruziziensis*, Mulato II (*U. ruziziensis* × *U. decumbens* (Stapf) R.D. Webster × *U. brizantha* (Hochst. ex A. Rich.) R.D. Webster) and *U. decumbens* cv. 'Basilisk' (Thaikua et al., 2018). Furthermore, agronomic evaluations at Lampang Animal Nutrition Research and Development Center demonstrated that cv. 'OKI-1' was tolerant of acidic soil, had a high leaf per stem ratio and fair productivity in terms of pure seed yield, averaging 209.6 kg/ha with a planting space of 150 × 150 cm. Consequently, both cv. 'OKI-1' and cv. 'Br-203' were considered to have considerable agronomic potential.

Seed yield is a trait of major interest in forage grass species as seed multiplication is economically important for new grass cultivars to become commercially viable (Boelt & Studer, 2010). Seed quality is a collective term that is used to describe the overall condition of the seeds, including aspects such as their genetic and physical purity, viability, vigor and seed health as these all play an important role in a competitive market. In order to produce good quality seeds, all aspects of the production system, i.e., from cultivation to agricultural management, storage and shipping need to be optimized (Elias, 2018).

Although cv. 'OKI-1' and cv. 'Br-203' can be established easily from seed, the principal constraint on its use and propagation is low seed availability and quality; this is also true for the other new hybrid *Urochloa* grasses, such as cv. 'Mulato', cv. 'Mulato II', cv. 'Cayman' and cv. 'Cobra' (Hare et al., 2015a). Successful seed production depends on several factors, including selection of planting site, climatic conditions, irrigation, soil fertility and cultivation practices. In addition, numerous studies have shown that proper crop management practices are the most promising factor for improving seed production. Of these crop management practices, plant spacing is one of the key factors affecting seed production (i.e., seed yield and seed quality) as it influences plant growth, especially the number of plant stems, which is related to the availability of nutrients and sunlight (Alem, 2021). The same grass cultivar can show differences in growth characteristics in different regions, even when cultivated using the same agricultural management methods. Previously, *Urochloa* grasses and some *Urochloa* hybrids showed the ability to produce high seed yields with good quality under proper cultivation and agricultural management in Southeast Asia, especially in Thailand. Thus, it is necessary to determine the optimal plant spacing for the new *Urochloa* grasses, cv. 'OKI-1' and cv. 'Br-203', which were selected for cultivation in this region. Grass stands seeded in rows provide the greatest seed yields and allow for easier cultivation and fertilizer application (Twidwell et al., 1987). Several studies have shown the advantages of row planting over broadcasting for grass seed production, mainly because of efficient utilization of sunlight, soil nutrients and water. Boonman (1972) showed that the seed yield of *Setaria sphacelata* cv. 'Nandi II' from broadcast sowing was lower than that for row planting using 50 cm-row widths and applying 100 kgN/ha. In addition, wider row spacing provides individual plants with more space to grow and makes weeding and harvesting easier, and decreases yield losses (Phaikaew et al., 2001).

The optimum plant density results in maximum seed yield, thus very low and very high densities reduce seed yields in grasses. (Kumar et al., 2005). For example, seed yield in *Urochloa brizantha* cv. MG 5 cultivated in Indonesia at a plant spacing of 100 × 100 cm was found to be higher than at plant spacings of 75 × 75 cm or 150 × 150 cm (354.43 kg/ha, 206.43 kg/ha and 128.87 kg/ha, respectively) (Umami et al., 2018). However, Joaquín et al. (2010) reported that the highest seed yield of *Urochloa brizantha* cv. 'Marandu' in Mexico was obtained using a spacing of 25 × 25 cm between rows and plants, and seed yield was higher than at a plant spacing of 50 × 50 cm, 75 × 75 cm, 100 × 100 cm, and 125 × 125 cm. In addition, Gbenou et al. (2019) showed that using 10,000 kg/ha goat manure and 33 kgNPK/ha as a fertilizer, the seed yield of *Panicum maximum* was higher at a row spacing of 60 cm than at 20, 40 and 80 cm.

Therefore, to consistently maximize seed yield by optimizing the quality of grass seed crops, it is essential to optimize row spacing to ensure an adequate population of plants for seed production (Gbenou et al., 2018), especially in a new plant line. The objective of this study was to determine the optimal plant spacing for seed production and related traits in the cv. 'OKI-1' and cv. 'Br-203' in Northern Thailand.

Materials and methods

Experimental site

Two field trials were conducted at the Lampang ANRDC, Lampang, Northern Thailand (18.3°N, 99°E; 320 m asl) from May 2018 to February 2020. The soil is classified as the Renu soil series, which is characterized by sandy loam to sandy clay loam soil, poor drainage, and low fertility (Land Development Department (LDD), 2020) as shown in Table 2-4.

Meteorological data

Figure 2-1(A), (B), and (C) depicts meteorological data from the Lampang ANRDC site in 2018 and 2019, including air temperature, rainfall, number of rainy days per year, relative humidity, sunshine duration per day, and day length. The average monthly air temperatures between 2018 and 2019 were similar, except for the period from April to July, but the amount of rainfall differed markedly. The annual rainfall in 2019 (864.5 mm with 93 rainy days) was less than that in 2018 (1,194.8 mm with 125 rainy days). The maximum rainfall in 2018 and 2019 occurred in July (242.8 mm) and August (330.1 mm), respectively, with no rain in January 2018 and February 2019. The mean temperature, sunshine duration, and relative humidity in 2018 were 26.6°C, 5.9 hours/day, and 79.5%, and in 2019 they were 27.4°C, 6.6

hours/day, and 71.6%, respectively. The average day length in both years was the same, 12 hours/day.

Experimental design

Trial 1 – Effect of plant spacing on seed yield and seed quality for cv. 'OKI-1'

In both 2018 and 2019, the trial was performed using RCBD with four inter- and intra-row spacings and four replicates. The inter- and intra-row spacings (and corresponding plant densities) were as follows:

T1 - spacing of 50 × 100 cm (2 plants/m^2)

T2 - spacing of 75 × 100 cm (1.3 plants/m^2)

T3 - spacing of 100 × 100 cm (1 plant/m^2)

T4 - spacing of 125 × 100 cm (0.8 plant/m^2)

Thirty-day-old seedlings of cv. 'OKI-1' were transplanted with the above spacing on June 23, 2018, using a plot size of 4 m × 5 m. A basal fertilizer dressing (312.5 kg/ha, NPK 15%: 15%: 15%) was applied with an additional application of 28 kgN/ha as urea at planting time. One week after transplantation, dead seedlings were replaced with fresh seedlings (prepared at the same time as the dead seedlings) to obtain a uniform stand. Two months later, each tussock was loosely tied to a bamboo stake using a plastic-coated wire to avoid expanding the growth cover over the ground. In the first year, the stand was established and no defoliation or cutting was performed. After the first seed harvest, the first cutting was performed (10 cm above ground level) to remove all the forage at the end of December 2018. The final cutting was performed on the July 15, 2019. Urea was applied at 28 kgN/ha immediately after final cutting.

Trial 2 – Effect of plant spacing on seed yield and seed quality for cv. 'Br-203'

The planting treatment and planting method, as well as the basal fertilizer application for cv. 'Br-203' were the same as those used for cv. 'OKI-1' in Trial 1.

Data collection and seed harvesting

In both trials, all seedheads within each plot, except for the border row, were harvested over the period of seed maturity (late November–early December). The TN/P was counted on three randomly selected plants in each plot at the time of harvest. IN/T was counted from 15 randomly selected tillers from the three selected plants. RN/I was counted from 15 randomly selected inflorescences of the three selected plants. SN/R was averaged from the upper-, mid-, and lower-racemes of each inflorescence, which was randomly selected from the 15 inflorescences of the same three selected plants. (SN/R were counted only in 2019). Tiller fertility was expressed as the percentage of inflorescences over the total number of tillers. All seedheads in each plot were tied together into manageable bunches when the seed was almost ripe. Nylon net bags were used to cover the bunch, and each bag was tied to a bamboo stake; the bag remained in place for the duration of the harvest. Bags were used to collect all the seeds produced. Ripe seed was threshed off the inflorescences by lightly tapping the nylon net bag. The plots were harvested individually.

Seed processing and calculation of secondary attributes

The seed was air-dried for several days in the shade, before being sun-dried for a few days and then cleaned using a hand screen and seed blower. SY was measured for each plot. SMC, TSW, PP and GP were determined following the test methods of the International Seed Testing Association for *Urochloa ruziziensis* (ISTA, 2011). TSW was determined from pure-seed spikelet weight. Purity analysis was performed by separating the pure seed fraction from inert matter and other seeds in each sample, and then separately weighing the pure seed fraction. The PP was calculated as the (weight of pure seed) × 100/(total weight of sample). The germination test was performed at 90 days after seed harvesting in each experimental year. SY was corrected to 11% SMC. FTP, TN, and IN, number of fertile tillers per plant (FTN/P), and FSP were calculated. The FTP was calculated as FTN × 100/TN, PSY was calculated as SY ×

PP/100, and PGSY was calculated as PSY × GP/100.

Statistical analysis

The data were analyzed and the estimated variance components in the RCBD and the treatment means were tested by the LSD using R (R Core Team, 2020).

Table 2-4 Chemical properties of initial soils of the experimental field in Trial 1 and Trial 2

Trial	pH	OM (%)	Total N (%)	Extractable (mg/kg)										EC (dS/m)
				P	K	Ca	Mg	Na	S	Mn	Fe	Cu	Zn	
1	5.04	0.59	0.03	2.58	20.46	30.18	3.54	4.83	2.39	12.92	84.04	0.15	0.10	0.34
2	5.28	0.61	0.03	1.01	57.35	58.56	10.18	10.41	3.49	9.14	51.13	0.32	0.02	0.37

Abbreviations: EC, electric conductivity; OM, organic matter.

Results

Seed yield and its components, as well as the seed quality for cv. 'OKI-1' are shown in Table 2-5 and Table 2-6, respectively. Although plant spacing had no significant effect on the seed yield of cv. 'OKI-1' in both years, an inter-row spacing of 100 cm tended to produce a higher PSY than the other spacings in the establishment year (2018) and the average of the two consecutive years. PSY was higher in 2018 than in 2019. Plant spacing was positively correlated with TN, FTN and IN, and negatively with the number of inflorescences per plant (IN/P) and FTP (Table 2-7). TN, FTN and IN were significantly affected by plant spacing, with the seed yield components being positively affected by closer plant spacing (Table 2-5). However, in the second harvest year of the experiment, the distance between rows of 75 cm tended to produce a higher PSY than the other spacings (Table 2-6). The trend in the seed yield components of TN, FTN, and IN/P this year was similar to the previous year.

Plant spacing had no significant effect on the overall seed quality for cv. 'OKI-1' in either of the years of the experiment (Table 2-6). In 2018, the average TSW, FSP, and GP values were 9.82 g, 31.87%, and 73.53%, respectively, while in 2019 they were 8.23 g, 24.88%, and 73.78%, respectively. A high PP value was observed at all plant spacings in both years (an average of 98.24%). For the two-year averages, FSP, PSY, and PGSY values at an inter-row spacing of 100 cm tended to be higher than at the other spacings.

Seed yield and its components and seed quality for cv. 'Br-203' are shown in Table 2-8 and Table 2-9, respectively. The effects of plant spacing on TSY, PSY and PGSY were not significantly different in 2018, and the average values obtained for TSY, PSY and PGSY were 238.91, 79.67, and 50.57 kg/ha, respectively. However, TN and FTN were significantly ($p < 0.05$) decreased at lower plant densities (wider spacing) as shown in Table 2-8. In 2018, IN/T was highest at an inter-row spacing of 100 cm and differed significantly from the other spacings. Plant spacing had no significant effect on IN, IN/P, RN/I, and FTP in both years (Table 2-8).

Even though plant spacing had no significant effect on TSY in the second harvest year (2019), PSY and PGSY were significantly ($p < 0.05$) higher at inter-row spacings of 75 cm, and this tendency was the same for the two-year average. However, the average values for TSY, PSY and PGSY in 2019 (196.81, 38.72 and 36.36 kg/ha, respectively) were lower than those obtained in 2018 (238.91, 79.67 and 50.57 kg/ha, respectively). In this trial, positive correlations were observed between plant density and TN (0.9997), FTN (0.9997), and IN (0.8843). In addition, IN/P was negatively correlated with RN/I (-0.9517) (Table 2-10).

In both years, FSP, PP, and GP were not significantly affected by plant spacing. The average FSP, PP, and GP were 14.24%, 96.72%, and 61.28%, respectively, in 2018, and 9.02%, 95.14%, and 92.31%, respectively, in 2019. However, TSW was the lowest at the widest plant spacing in the first harvest year and tended towards being the lowest at the same spacing in the second harvest year (Table 2-9).

Table 2-5 Effect of plant spacing on seed yield and seed yield components in cultivar ‘OKI-1’

Year	Plant spacing (cm × cm)	Plant density (no./m^2)	TSY (kg/ha)	TN (no./m^2)	FTN (no./m^2)	IN/P (no./plant)	IN (no./m^2)	FTP (%)	IN/T (no.)	RN/I (no.)	SN/R (no.)
2018	50 × 100	2	285.42	64.67^{a}	45.17^{a}	63.05	126.10^{a}	79.66	2.65	3.43	-
	75 × 100	1.3	216.67	41.60^{b}	40.84^{ab}	64.93	84.37^{ab}	86.27	2.38	3.52	-
	100 × 100	1	292.75	36.25^{b}	31.00^{b}	98.97	98.97^{ab}	86.41	3.15	2.92	-
	125 × 100	0.8	276.67	34.67^{b}	30.73^{b}	99.34	79.47^{b}	88.94	2.53	3.66	-
2019	50 × 100	2	164.58	76.67^{a}	70.67^{a}	37.85^{b}	75.70	93.78	1.07	4.82	36.92
	75 × 100	1.3	144.70	56.66^{ab}	54.06^{ab}	44.08^{ab}	57.31	94.98	1.08	5.17	35.96
	100 × 100	1	107.66	55.17^{ab}	51.83^{ab}	59.05^{ab}	59.05	93.92	1.17	4.47	35.48
	125 × 100	0.8	103.96	45.60^{b}	43.47^{b}	63.08^{a}	50.47	95.52	1.17	4.69	37.74
2-year average	50 × 100	2	225.00	70.67^{a}	57.92^{a}	50.45	100.90^{a}	86.72	1.86	4.12	-
	75 × 100	1.3	180.69	49.13^{b}	47.45^{ab}	54.50	70.84^{b}	90.63	1.73	4.34	-
	100 × 100	1	200.20	45.71^{b}	41.42^{b}	79.01	79.01^{ab}	90.16	2.16	3.69	-
	125 × 100	0.8	190.28	40.13^{b}	37.10^{b}	81.21	64.97^{b}	92.23	1.85	4.17	-

Abbreviations: FTN, fertile tiller number per square meter; FTP, fertile tiller percentage; IN, inflorescence number per square meter; IN/P, inflorescence number per plant; IN/T, inflorescence number per tiller; RN/I, raceme number per inflorescence; SN/R, spikelet number per raceme; TN, tiller number per square meter; TSY, total seed yield. Note: Values with different superscript letters indicate statistical differences within each column for each year or the 2-year average ($p < 0.05$).

Table 2-6 Effect of plant spacing on seed quality in cultivar 'OKI-1'

Year	Plant spacing (cm × cm)	Plant density (no./m^2)	FSP (%)	TSW (g)	PP (%)	PSY (kg/ha)	GP (%)	PGSY (kg/ha)
2018	50 × 100	2	21.21	9.43	92.82	147.31	86.25	127.69
	75 × 100	1.3	32.68	10.06	98.62	154.32	70.44	116.78
	100 × 100	1	40.57	9.96	98.80	208.84	69.06	146.27
	125 × 100	0.8	33.01	9.83	99.00	184.55	68.38	116.19
2019	50 × 100	2	16.3	8.09	99.55	84.98	70.12	57.01
	75 × 100	1.3	28.37	8.44	99.55	89.81	65.12	57.64
	100 × 100	1	27.37	8.44	99.12	64.07	83.88	52.18
	125 × 100	0.8	27.46	7.94	98.48	62.72	76.01	43.92
2-year average	50 × 100	2	18.76	8.76	96.19	116.15	78.19	92.35
	75 × 100	1.3	30.53	9.45	99.09	122.07	67.78	87.21
	100 × 100	1	33.97	9.20	98.96	136.46	76.47	99.22
	125 × 100	0.8	30.24	8.88	98.74	123.63	72.20	80.05

Abbreviations: FSP, filled seed percentage; GP, germination percentage; PGSY, pure germinated seed yield; PP, purity percentage; PSY, pure seed yield; TSW, thousand seed weight.

Table 2-7 Correlation coefficients among seed components and plant density in cultivar 'OKI-1' (2-year average)

	Plant density	TN	FTN	IN/P	IN	FTP	IN/T
TN	0.9893**						
FTN	0.9961**	0.9748**					
IN/P	-0.8835*	-0.8057	-0.9126*				
IN	0.9007*	0.9486*	0.8778	-0.6063			
FTP	-0.9484*	-0.9751**	-0.9359*	0.7142	-0.9888**		
IN/T	-0.2807	-0.1772	-0.2963	0.5891	0.1406	-0.0360	
RN/I	0.2295	0.1250	0.2456	-0.5490	-0.1924	0.0888	-0.9986**

Abbreviations: FTN, fertile tiller number per square meter; FTP, fertile tiller percentage; IN, inflorescence number per square meter; IN/P, inflorescence number per plant; IN/T, inflorescence number per tiller; RN/I, raceme number per inflorescence; TN, tiller number per square meter. *Correlation is significant at the 0.05 level (two-tailed), $p < 0.05 = 0.8783$. **Correlation is significant at the 0.01 level (two-tailed), $p < 0.01 = 0.9587$. Note: Values without asterisks are not significant.

Table 2-8 Effect of plant spacing on seed yield and seed yield components in cultivar 'Br-203'

Year	Plant spacing (cm × cm)	Plant density (no./m^2)	TSY (kg/ha)	TN (no./m^2)	FTN (no./m^2)	IN/P (no./plant)	IN (no./m^2)	FTP (%)	IN/T (no.)	RN/I (no.)	SN/R (no.)
2018	50 × 100	2	268.59	103.00^{a}	64.33^{a}	94.90	189.81	73.49	2.97^{ab}	3.55	-
	75 × 100	1.3	235.92	81.68^{ab}	58.72^{ab}	121.53	157.99	71.46	2.75^{b}	4.06	-
	100 × 100	1	238.26	66.42^{b}	45.75^{ab}	171.51	151.71	68.06	3.70^{a}	3.07	-
	125 × 100	0.8	212.87	56.67^{b}	41.93^{b}	160.95	128.76	74.60	3.08^{b}	3.07	-
2019	50 × 100	2	225.17	136.83^{a}	128.67^{a}	200.57	401.13	93.64	1.47	4.24	33.43
	75 × 100	1.3	250.42	92.30^{b}	84.07^{b}	159.67	207.57	90.92	1.48	4.16	33.78
	100 × 100	1	142.98	78.00^{b}	72.42^{b}	256.25	256.25	92.26	1.85	3.62	34.29
	125 × 100	0.8	168.65	66.40^{b}	61.80^{b}	278.47	222.77	93.46	1.53	3.96	34.87
2-year average	50 × 100	2	246.88	119.92^{a}	96.50^{a}	147.74	295.47^{a}	83.57	2.22^{b}	3.90	-
	75 × 100	1.3	243.17	86.99^{b}	71.39^{b}	146.60	182.78^{b}	81.19	2.12^{b}	4.11	-
	100 × 100	1	190.76	72.21^{bc}	59.08^{bc}	213.88	213.88^{ab}	80.16	2.78^{a}	3.34	-
	125 × 100	0.8	190.62	61.53^{c}	51.87^{c}	219.71	175.76^{b}	84.30	2.31^{ab}	3.51	-

Abbreviations: FTN, fertile tiller number per square meter; FTP, fertile tiller percentage; IN, inflorescence number per square meter; IN/P, inflorescence number per plant; IN/T, inflorescence number per tiller; RN/I, raceme number per inflorescence; SN/R, spikelet number per raceme; TN, tiller number per square meter; TSY, total seed yield. Note: Values with different superscript letters indicate statistical differences within each column for each year or the 2-year average (p < 0.05).

Table 2-9 Effect of plant spacing on seed quality in cultivar 'Br-203'

Year	Plant spacing (cm × cm)	Plant density (no./m^2)	FSP (%)	TSW (g)	PP (%)	PSY (kg/ha)	GP (%)	PGSY (kg/ha)
2018	50 × 100	2	9.60	6.78^{a}	95.95	69.58	56.06	45.48
	75 × 100	1.3	17.59	6.69^{a}	95.88	87.96	58.06	50.06
	100 × 100	1	15.88	7.01^{a}	97.68	96.36	69.19	67.52
	125 × 100	0.8	13.88	6.06^{b}	97.35	64.78	61.81	39.22
2019	50 × 100	2	6.04	6.80	96.80	33.41^{ab}	89.75	30.75^{ab}
	75 × 100	1.3	14.76	6.77	96.95	71.22^{a}	94.75	68.12^{a}
	100 × 100	1	8.93	6.25	94.75	29.99^{ab}	92.25	27.99^{ab}
	125 × 100	0.8	6.33	5.72	92.08	20.24^{b}	92.50	18.60^{b}
2-year average	50 × 100	2	7.82	6.79^{a}	96.38	51.50	72.91	38.11
	75 × 100	1.3	16.17	6.73^{a}	96.41	79.59	76.41	59.09
	100 × 100	1	12.40	6.63^{a}	96.21	63.17	80.72	47.76
	125 × 100	0.8	10.11	5.89b	94.71	42.51	77.16	28.91

Abbreviations: FSP, filled seed percentage; GP, germination percentage; PGSY, pure germinated seed yield; PP, purity percentage; PSY, pure seed yield; TSW, thousand seed weight. Note: Values with different superscript letters indicate statistical differences within each column for each year or the 2-year average ($p < 0.05$).

Table 2-10 Correlation coefficients among seed components and plant density in cultivar 'Br-203' (2-year average)

	Plant density	TN	FTN	IN/P	IN	FTP	IN/T
TN	0.9997**						
FTN	0.9997**	0.9997**					
IN/P	-0.7923	-0.8009	-0.8078				
IN	0.8843*	0.8793*	0.8720	-0.4221			
FTP	0.1729	0.1517	0.1676	-0.0392	-0.1924		
IN/T	-0.4328	-0.4293	-0.4499	0.7106	-0.0420	-0.5321	
RN/I	0.6202	0.6270	0.6400	-0.9517*	0.1830	0.0916	-0.8671

Abbreviations: FTN, fertile tiller number per square meter; FTP, fertile tiller percentage; IN, inflorescence number per square meter; IN/P, inflorescence number per plant; IN/T, inflorescence number per tiller; RN/I, raceme number per inflorescence; TN, tiller number per square meter. *Correlation is significant at the 0.05 level (two-tailed), $p < 0.05 = 0.8783$. **Correlation is significant at the 0.01 level (two-tailed), $p < 0.01 = 0.9587$. Note: Values without asterisks are not significant.

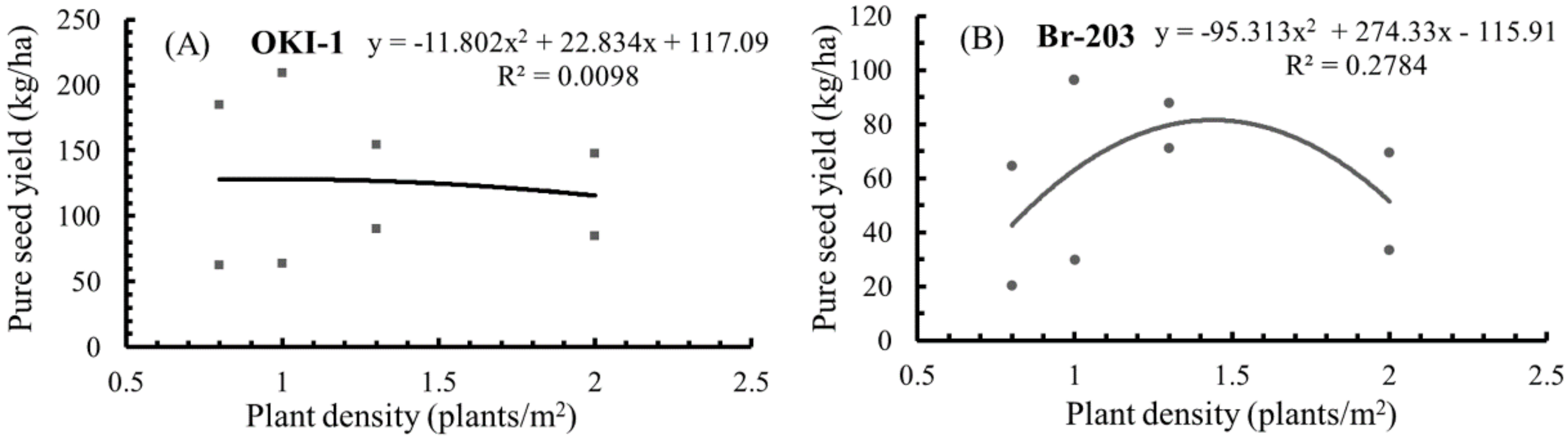

Figure 2-2 Relationships between plant density and pure seed yield in cultivars cv. 'OKI-1' (A) and cv. 'Br-203' (B).

Discussion

Conditions at experimental sites

Matching the forage species to the production areas is important for optimizing seed production, because conditions such as climate, photoperiod, and soil fertility all have a major effect on shoot density, the number of seeds formed per flower, and the percentage harvest recovery of the seed, which are three key traits associated with seed yield (Gbenou et al., 2018). Rao et al. (1996) reported that most commercial *Urochloa* spp. are adapted to a wide range of soil types; from poor acidic soils to highly fertile neutral soils. Despite the fact that the soils at both trial sites were acidic, with low organic matter (OM) content (< 1%) and deficiencies in essential nutrients, the cultivars 'OKI-1' and 'Br-203' demonstrated edaphic adaptation in terms of growth and productivity (Land Development Department (LDD), 2010) (Table 2-4).

Conversely, increasing seed production in *Urochloa* spp. depends primarily on their adaptability to the climate. Weather conditions, especially rainfall, greatly influenced seed set and pure live seed yield (Boonman, 1973). *Urochloa* spp. are generally grown in higher rainfall areas (> 2,000 mm/year), but they have been used in areas with a minimum annual rainfall of approximately 1,000 mm in Australia (Stür et al., 1996), and in some *Urochloa* hybrids, even in areas with an annual rainfall as low as 700 mm and a dry season of 5–6 months (Kendrick, 2008). The role of temperature in seed production is as important as rainfall. Bouathong et al. (2011) reported that the optimum temperature and humidity for *Urochloa* spp. were 25–35°C and 60–70%, respectively. In a report by Nadew (2018), the roles of rainfall and temperature were shown to be key climatic conditions that markedly affect the state of the physiological processes in seeds, and consequently, the yield and quality of seeds. Bouathong et al. (2011) reported that the monthly rainfall required by *Urochloa* grass throughout its growth period is 83–125 mm. Loch (1980) stressed the importance of the amount and distribution of rainfall and how these factors affect seed yields, particularly during inflorescence emergence.

Possible reasons for the decrease in the seed yields for cv. 'OKI-1' and cv. 'Br-203' in 2019, compared to values in 2018, might be water shortage, low relative humidity during seed development and maturation (October–November), and the low water-holding capacity and high drainage rate of the sandy loam soil at the experimental sites. These factors may have resulted in earlier plant senescence, a more marked decline in seed moisture content and seed quality development, and the premature death of spikelets. In other words, rapid growth to maturity at high temperatures during anthesis can reduce seed fertility and yield. In both cultivars, the start of flowering was three days apart in both years of the experiment (cv. 'OKI-1': October 18, 2018 vs. October 15, 2019 and cv. 'Br-203': October 19, 2018 vs. October 16, 2019). The seeds produced in 2019 ripened early, and defects in the seed filling process were observed. In addition, the distribution of rainfall in 2018 was better than that in 2019 (Figure 2-1).

Compared to the study of Phunphiphat et al. (2007), which was conducted in the same area as the experiments of this study, the average PSY values of Mulato and Mulato II in two consecutive years (2004–2005) were higher than both cv. 'OKI-1' and cv. 'Br-203'. In their study, the average annual rainfall was 1,239 mm and the distribution of rainfall was better than that in the period 2018–2019, especially during the period of vegetative growth. Moreover, in 2019, there was almost no rain in October and November, which are critical months for seed maturation. Finally, the growing period for the plants in 2018 was longer than that in 2019 because no defoliation was performed; as a result, the seed yield in 2018 was higher than that in 2019.

In addition to the aforementioned factors, the sufficiency of solar radiation and favorable photoperiods at each stage of the growth cycle, including the periods of dry weather during maturation and harvest, also markedly affected seed production. At the experimental sites, the average day length throughout the experimental period was approximately 12 hours/day (11 hours/day in December to 13 hours/day in June), and the duration of sunshine averaged 6 hours/day, with the shortest duration in August (approximately 3 hours/day) and the longest duration in April (approximately 9 hours/day). These conditions were likely sufficient for growth and flowering of *Urochloa* cultivars and corroborate the findings of Wongsuwan (1994), who found that a critical day length triggers reproductive development of *Urochloa ruziziensis* in Thailand (approximately 12 ½ hours) and that the greatest seed yield was obtained under conditions of shorter day length (11 hours) than under longer day lengths (14, 13 and 12 hours). Therefore, material for increasing seed yield must be selected to match the climatic conditions, day length, and soil.

Trial 1 – Effect of plant spacing on seed yield and seed quality for cv. 'OKI-1'

While plant spacing caused a significant increase in the tiller and inflorescence densities of cv. 'OKI-1' (Table 2-5), it did not significantly affect seed yields and/or seed quality (Table 2-6). These findings showed that the highest TN, FTN, and IN values were obtained using the narrowest plant spacing (50 × 100 cm). Compared with the other plant spacings tested, IN/T was highest at a plant spacing of 100 × 100 cm. However, RN/I tended to be the lowest. Wider plant spacing increased IN/P in compensation for decreased TN and IN. These results corroborate the findings of Phaikaew et al. (2001), who demonstrated that a narrower plant spacing induced significantly more tillers and inflorescences than wider plant spacings. In a study on the seed production potential of *Urochloa* spp. in Northeast Thailand, Nakmanee & Phaikaew (1998) also reported that the inflorescence density was not always associated with high seed yields. Likewise, Kamphayae et al. (2013) reported that the pure seed yield for *Panicum maximum* Jacq. cv. 'Umaku' at the wider plant spacings tended to be higher than at narrower spacings, which had the effect of decreasing the size of inflorescences. These authors proposed that the low seed yield might be due to a failure in seed-set caused by an increase in the competition for soil nutrients at high plant densities, even though the inflorescence density decreased with increasing plant spacing.

In a genetic assessment of seed yield-related traits in *Paspalum* spp., Lopes et al. (2019) suggested that the number of seeds/inflorescence and reproductive tillers/plant was strongly correlated with seed yield/plant and that these traits could be used for indirect selection of seed yield in crosses of *P. plicatulum* Micchx. × *P. guenoarum* Arechav. However, Gallagher & Biscoe (1978) showed that even though tillering is an important agronomic trait in graminaceous plants as it plays an important role in determining seed yield, not all tillers produce spikelets and numerous tillers abort before anthesis. In addition, high seed yields are dependent on maximizing the number of tillers that synchronously produce inflorescences (Loch, 1985), and although it is important for seed crops to have a high number of fertile tillers at seed harvest, this does not necessarily equate to a high total tiller number. Moreover, the measurement of tiller number can be conducted at different developmental stages. For example, assessments of tiller number during the vegetative stages provide an estimate of maximum tillering, while assessments of tiller number at or after heading provide an estimate of productive tiller number (Naruoka et al., 2011).

In this study, the PSY value for cv. 'OKI-1' was high in the first year (2018) and low in the following year (i.e., averaging 174 kg/ha and 75 kg/ha, respectively); even though the number of tillers at all plant spacings was increased. This is probably because soil nutrients declined and climatic conditions, especially temperature, may have been higher during flowering and seed setting. Numerous shriveled seeds were obtained in the second year of the experiment (2019). In addition, seed integrity for all plant spacing tended to be higher in the first year than in the second year, and the lowest FSP was obtained at a plant spacing of 50 × 100 cm in both years. The results also showed that a maximum TSW was not achieved at either the widest or narrowest plant spacings. According to Allen & Prasad (2004), crops are highly sensitive to an increase in temperature from several days before pollen maturation through fertilization of the ovule and during seed-filling processes. For example, they found that soybean and kidney bean seeds failed to fill properly as the temperature increased, and that plants subjected to increased temperatures during this sensitive period formed smaller, shriveled seeds. However, the average PSY of cv. ‘OKI-1’ in this two-year experiment was unaffected by different plant spacings; although PSY was highest (136.46 kg/ha) at 100 × 100 cm, compared to spacings of 125 × 100, 75 × 100, and 50 × 100 cm (i.e., PSY was 123.64, 122.06, and 116.14 kg/ha, respectively).

Although plant spacing had no significant effect on seed yield in cv. ‘OKI-1’ in this study, the lowest seed yield tended to be observed at a plant spacing of 50 × 100 cm. Consequently, to minimize production costs and limit other management expenditures, a plant spacing of 50 × 100 cm is not recommended for this cultivar.

Trial 2 – Effect of plant spacing on seed yield and seed quality for cv. ‘Br-203’

The effects of plant spacing on TN, FTN, IN, and IN/T varied. The narrowest plant spacing (50 × 100 cm) had more tillers per unit area than the wider plant spacings, but the highest number of inflorescences was found at a plant spacing of 100 × 100 cm. Plant density was positively correlated with tiller and inflorescence density, as shown in Table 2-10. In the first year of the experiment, plant density did not show any tendencies in terms of TSY, PSY, or PGSY. However, in the second year of the experiment, plant density had a significant effect on PSY and PGSY, with plant spacings of 75 × 100 cm and 125 × 100 cm associated with the highest and lowest seed yield, respectively. Furthermore, as for cv. ‘OKI-1’, the seed yield for cv. ‘Br-203’ in 2019 was less than that in 2018. An increase in the number of tillers in 2019 had no influence on the increase in seed yield. Boelt & Studer (2010) reported that the number of reproductive tillers per unit area is important for seed yield potential, but that this varies among grass species. They also showed that the most effective tillers emerge before or during primary floret induction, and that this timing affects the likelihood of becoming reproductive.

The FSP for cv. ‘Br-203’ in both years was very low, with that for the second-year crops tending to be lower than that for the first-year crops; the lowest FSP was observed at a plant spacing of 50 × 100 cm, and the highest FSP at a plant spacing of 75 × 100 cm. Plant spacing had a significant effect on TSW in first-year crops and the average of both years; the smallest TSW was at the widest plant spacing (125 × 100 cm). In addition, seeds from the second-year crop had markedly higher germination rates compared to the first-year crops, and a tendency towards the lowest rates of seed germination was observed at the narrowest plant spacing.

Although plant spacing did not have a significant effect on total and pure seed yields, a tendency towards a higher seed yield and improved seed quality was observed at a plant spacing of 75 × 100 cm, compared to the other plant spacings. Consequently, to maintain seed yield in the years after establishment, an optimum row spacing of 75 to 100 cm (1.0–1.3 plants/m^2) is recommended. It is considered that using this plant density will reduce production costs and optimize cultivation and management practices.

A general yield-density relationship is known to exist in crops grown for grain or seed. Silvertown (1987) reported that this relationship in crops such as maize, wheat, and ryegrass grown for seed can best be depicted by a parabolic yield curve. In these crops, total plant weight is typically asymptotically related to density, but the yield from reproductive parts of the plant increases to reach its maximum and then gradually decreases again as the population density increases.

In this study, the seed yield curve (i.e., TSY or PSY) could best be depicted by an upward convex-shaped graph for cv. ‘OKI-1’, but that this convex was more apparent for cv. ‘Br-203.’ However, both cultivars tended to achieve maximum seed yields with seeds of good quality under an optimum plant density of 1–1.3 plants/m^2, which was shown to be suitable for a plant spacing of 100 × 100 cm and 75 × 100 cm (Figure 2-2).

As the density at which the maximum seed yield can vary with the nutrient status of the plants (Willey & Heath, 1969), further research is considered necessary.

Conclusions

This study investigated seed yield, seed quality, and seed yield components of new *Urochloa* cultivars, cv. ‘OKI-1’ and cv. ‘Br-203’, under different plant spacings over two consecutive years in Northern Thailand. The results showed that the seed yield components of cv. ‘OKI-1’ and cv. ‘Br-203’, particularly the number of tillers and inflorescences, are affected by plant spacing. Although the plant spacing did not have a significant effect on seed yield, both cultivars tended to produce the maximum seed yield with good quality at an optimum plant spacing, which for cv. ‘OKI-1’ and cv. ‘Br-203’ was 100 × 100 cm and 75 × 100 cm, respectively.

Effects of closing cut date and nitrogen fertilization on seed yield and seed quality in two novel cultivars of *Urochloa* spp.

Abstract

Two field trials were conducted in Thailand to determine an appropriate CCD and rate of nitrogen application (N-rate) to maximize seed yield and seed quality of the two novel cultivars (cv.) of *Urochloa* spp. (syn. *Brachiaria* spp.), cv. 'OKI-1' (an open-pollinated tetraploid *Urochloa ruziziensis* (R. Germ. and C.M. Evrard) Crins originated from cv. 'Miyaokikoku-ichigou') and cv. 'Br-203' (*U. ruziziensis* cv. 'Miyaokikoku-ichigou' × *U.* hybrid cv. 'Mulato'). The following treatments were evaluated in this study: four CCDs (uncut, 15 June, 1 July, and 15 July) and four N-rates (0, 50, 100, and 150 kg/ha). The cv. 'OKI-1' showed somewhat differences in TN, FTP, IN/T and SN/R with the CCD, while the cv. 'Br-203' showed only in SN/R. However, TN and SN/R were highest for 15 June, and FTP and IN/T were highest for 1 July in cv. 'OKI-1'. The cv. 'OKI-1' showed the highest TSY, PSY, and PGSY for 1 July, followed by 15 June, and the cv. 'Br-203' showed the highest TSY, PSY, and PGSY for 15 July, followed by 1 July. N fertilization showed a negative effect on TSY for both the cultivars due to the higher N content in the soil. Withholding N fertilizer, a CCD in late-June to early-July and early-July to mid-July is recommended for cv. 'OKI-1' and cv. 'Br-203', respectively.

Introduction

Urochloa (synonym *Brachiaria*) is the most extensively distributed genus used for forage grass in the tropics, with approximately 100 species, including several commercially important species (Fisher & Kerridge, 1996). Ruminant livestock farmers use *Urochloa* spp. because of their high forage production and palatability for cattle, as well as their high nutritive value, adaptability, and environmental benefits (e.g., reducing soil erosion and greenhouse gas emissions) (Rao et al., 2015). *Urochloa* spp. can be utilized as pasture, fodder in cut-and-carry systems, hay, silage, and mixed grass-legumes, among other things. Grasses from the genus *Urochloa* have been adopted to alleviate livestock feed shortages and improve the availability and quality of feeds. Furthermore, it can increase livestock productivity and farmers' revenue (Cheruiyot et al., 2020).

In this study, the optimal N-rate and timing of CCD were investigated in two novel candidate cultivars of *Urochloa* grass (syn. *Brachiaria* grass). The one candidate cultivar (cv.) 'OKI-1', is an open-pollinated cultivar (Ishigaki et al., 2009a) that originated from recurrent selection through the evaluation of seed fertility and plant productivity among the base population of tetraploid *Urochloa ruziziensis* (R. Germ. and C.M. Evrard) Crins. cv. 'Miyaokikoku-ichigou' (Kouki et al., 2014). The cv. 'OKI-1' was obtained by the DLD, Thailand after pre-selection of the desired traits in Japan. The other candidate, cv. 'Br-203', is an apomictic hybrid produced by crossing a sexual maternal tetraploid parent of cv. 'Miyaokikoku-ichigou' (*U. ruziziensis*) (Kouki et al., 2014) with an apomictic paternal tetraploid parent of cv. 'Mulato' (*U. ruziziensis* × *U. brizantha* (Hochst. ex A. Rich.) Stapf). The cv. 'OKI-1' had significantly higher IVDMD compared with *U. ruziziensis*, cv. 'Mulato II' (*U. ruziziensis* × *U. decumbens* (Stapf) R.D. Webster × *U. brizantha*) and cv. 'Basilisk' (*U. decumbens*) (Thaikua et al., 2018). In addition, the cv. 'Br-203' had a high forage DM production (19,200 kg/ha/year on average over two years at five test sites in Thailand), excellent regrowth and extension, high palatability, relatively frequent flowering, and a seed ripening rate comparable to 'Basilisk' (Nakmanee et al., 2015). In a preliminary trial at the Lampang ANRDC in 2017, cv. 'OKI-1' produced 15,800 kg/ha/year of forage DM yield. Widespread adoption of these cultivars requires that they are in demand and that they can be produced economically by increasing seed yield under good agronomic management.

The purpose of agricultural management for tropical grass seed production is to produce a synchronized, high-yielding seed crop by encouraging inflorescence development and restricting this development to a short emergence duration (Loch, 1999a); the closing cut is an important agronomic practice that is used to accomplish these aims. To enable the crop to flower properly, the closing cut should be scheduled carefully. Closing cuts that are too early will produce an excessive bulk of tall grass at harvest, which can increase the risk of lodging, disease occurrence, insect infestation, hinder inflorescence emergence and harvesting, and increase seed shattering losses (Hare et al., 2007b; FAR, 2016). On the other hand, closing cuts that are too late will remove many developing reproductive apices (Hare et al., 1999), ultimately decreasing inflorescence numbers and seed yields. In addition, the decreased leaf area from late regrowth can reduce seed yields due to a small inflorescence, even though the grass may be flowering abundantly when cut too late (Gorbius et al., 1998).

Hare et al. (2007b) reported that the final CCD is also very important for seed production in *Urochloa* hybrids in Thailand. The optimal time to carry out a final closing cut on the seed crops of cv. 'Mulato' and cv. 'Mulato II' was during July (mid-wet season). Optimal times for the closing cut have also been determined for *U. ruziziensis* (Phaikaew & Pholsen, 1993) and cv. 'Mulato' (Nakmanee et al., 2007b) in Thailand. These studies, which were conducted in Northeast Thailand, recommend that the time of the final closing cut should not be delayed past July, as the harvesting period of these cultivars is mid-November to early December. In addition, early closing cuts (May–June) and late closing cuts (September) either decreased seed yields markedly or resulted in no seeds being produced at all (Phaikaew et al., 1985).

N is a major factor limiting the production of grass seeds. Because N plays such a central role in determining grass seed yield, a key decision is when and how much N fertilizer should be applied (Hare et al., 2007b). The appropriate N-rate to optimize seed yield and quality in *Urochloa* hybrids (Bouathong et al., 2011), cv. 'Mulato' (Nakmanee et al., 2007b), *U. ruziziensis* (Satjipanon et al., 1987; Wongsuwan, 1999), and *Paspalum atratum* (Phaikaew et al., 2002) has been determined in Thailand. These studies found that the optimal N-rate varied from 40 kg/ha to 400 kg/ha due to several factors, such as soil nutrients, including N, soil moisture, changes in environmental parameters that affect crop development, and other aspects related to crop management. Research groups in different countries have undertaken studies on *Urochloa*; for example, in Brazil, studies have been conducted on *U. brizantha* (Benteo et al., 2016) and *U. decumbens* (Canto et al., 2020), and in Benin, on *U. ruziziensis* (Adjolohoun et al., 2013). Their results showed that N-rates for seed production ranged from 50 kg/ha to 100 kg/ha.

The relationship between the CCD and the N-rate has been investigated in *U. ruziziensis* by Wongsuwan (1999), who found that a high pure germinated seed yield (PGSY) could be obtained using an N-rate of 150 kg N/ha and an early CCD (24 March 1997). Satjipanon et al. (1987) reported that high seed yield with good quality was obtained in *U. ruziziensis* using a CCD on 14 August 1984 and 7 August 1985 with an N-rate of 400 kg N/ha. In other grass cultivars, such as cv. 'Mulato', the optimal CCD should not be later than 15 July, and N fertilizer should be applied at 100 kg N/ha thereafter (Nakmanee et al., 2007b).

Successful seed production of the two novel cultivars of *Urochloa* spp., cv. 'OKI-1' and cv. 'Br-203', could provide the basis for greatly increased forage production. To maximize seed production, an understanding of cultivation practices was required for widespread adoption of the new cultivars. With the knowledge of cultivation practices, including an optimal CCD and N-rate, seed producers can decide whether such an enterprise would be technically and economically viable. In addition, to provide useful information on the agricultural management of these grasses when they are promoted for planting in Thailand, two trials were designed to evaluate the effects of N-rate and CCD on seed yield and seed quality of both cultivars. They were examined under four CCDs (uncut, 15 June, 1 July, and 15 July) and four N-rates (0, 50, 100, and 150 kg N/ha) with four replications in a split-plot configuration in RCBD.

Materials and methods

Location of the Experiment

From May 2018 to February 2020, two field experiments were carried out at the Lampang ANRDC, Hang Chat, Lampang, Northern Thailand (18.3° N, 99° E; 320 m asl). The soil belongs to the Renu soil series (Land Development Department (LDD), 2009). This soil has been classified according to the United States Department of Agriculture (USDA) soil taxonomy as Ultisols, with sandy loam in the topsoil and sandy clay in the subsoil. The preliminary soil and post-harvest soil samples were collected at a depth of 0–15 cm.

Meteorological Data

In 2018 and 2019, data on temperature, precipitation, rainy days, sunshine duration, relative humidity, and day length at the Lampang ANRDC were collected from the Hang Chat Agrometeorological Station in Hang Chat, Lampang, Thailand, which is about 2 km away from the experimental sites.

Experimental Design and Treatments

Trial 1: Effect of CCD and N-rate on seed yield, seed yield components, and seed quality in the candidate cultivar 'OKI-1'

The trial was conducted using a split-plot configuration in RCBD with four replicates (blocks). The main-plot treatments comprised four CCD treatments, i.e., uncut, cut on 15 June, 1 July, and 15 July, and sub-plot treatments within each main-plot included N-rates of 0, 50, 100, and 150 kg N/ha applied in the form of urea (46% N).

On 22 June 2018, the 30-day-old seedlings of cv. 'OKI-1' were planted in a plot size of 4 × 5 m at a spacing of 1 × 1 m. At the time of planting, a basal fertilizer dressing (312.5 kg NPK/ha, 15%:15%:15%) was applied. To maintain uniform stands, any dead seedlings were replaced with fresh seedlings (grown at the same time as the deceased seedlings) within one week after planting. Two months later, each tussock was loosely tied to a bamboo stake with plastic-coated wire to prevent its growth covering the ground. Weeds were controlled by removing them manually over the course of the experiment. The establishment year (2018) data were not analyzed or reported because all seedlings were planted on 22 June. After seed harvest, the grass cutting was performed on 28 December 2018 at 10 cm above ground level. The plant residues were then manually removed within one day after cutting and topdressing fertilizers (62.5 kg P/ha as single super phosphate and 62.5 kg K/ha as potassium chloride) were applied immediately. The effect of the CCD and N-rate was then analyzed in 2019 (the second year of the experiment). The closing cut occurred at the time specified, and the grass was cut with a sickle, as shown in Figure 2-3. After the closing cut of each treatment, topdressing with urea at a predefined rate was applied immediately to each tussock.

Trial 2: Effect of CCD and N-rate on seed yield, seed yield components, and seed quality in a cv. 'Br-203'

The experimental site used for this trial was approximately 30 m away from the experimental site of Trial 1. For cv. 'Br-203', all the treatments and methods, and the basal fertilizer rate used were the same as those applied for cv. 'OKI-1' in Trial 1.

Data Collection and Seed Harvesting

In all two trials, the beginning of the first flowering time was recorded for three randomly selected plants per plot once the first inflorescence had fully emerged from the sheath. Except for the border row, all the inflorescences within each plot were harvested when the seeds were mature (late November to late December). At the time of harvest, three randomly selected plants in each plot were counted for the TN/P. Fifteen randomly selected tillers on the three selected plants were counted for the IN/T. Fifteen randomly selected inflorescences on the three selected plants were counted for the RN/I. SN/R was calculated by averaging the top, mid, and lower racemes of each inflorescence, which were chosen at random from among the fifteen inflorescences on the same three selected plants. Tiller fertility was calculated as the percentage of fertile tillers over the total number of tillers. When the seed was almost ripe, all the inflorescences in each tussock were tied together as manageable bunches. The mature seeds were then shed from the manageable bunches and gathered with the use of a nylon net sheet positioned beneath the inflorescences. As shown in Figure 2-4, three fine 20-mesh nylon net sheets per plot were used. The two nylon net sheets (A) each have a width of 1.2 m and a length of 5 m. The other one is a nylon net sheet (B) that is 1.2 m in width and 3 m in length. The bunches of grass plants, especially the inflorescences, were bent towards the nylon net sheets, following the red arrow directions. The experimental plots during seed harvesting are shown in Figure 2-5. When harvesting seeds, ripe seeds are threshed off the inflorescence by gently tapping. The ripened seeds fall into the nylon net sheet. The fallen seeds were scooped from the nylon net sheets and placed in two fine mesh nylon bags prepared for collecting seeds in each plot for easy air flow and sunlight drying. One bag was used for collecting seeds from two "A" nylon net sheets (border rows of grass plants), and the other bag was used for collecting seeds from a "B" nylon net sheet (six plants inside the plot). Only the collected seed from the "B" nylon net sheet in each plot was used to estimate seed yield, to avoid edge effects and bias caused by abiotic environmental factors that affected each grass plant (i.e., sunlight, wind, humidity). All bags of grass seed were sun-dried on the ground and hung for a few days after being hung for several days in the shade. Seeds were collected every four days except for the final collection, which was collected 15 days after the previous collection (a total of five times). The plots were harvested separately.

Seed Processing and Calculation of Secondary Attributes

The harvested seeds were air-dried in the shade for several days before being sun-dried for a few days and then cleaned with a hand screen. Seeds with endosperm (filled seeds) were separated from the inert fraction and empty or light seeds using a South Dakota seed blower. The seed blower works by using airflow generated by a blower motor, and as that air passes through a column, the air lifts light, fluffy material or empty seed into the top beveled area of the column, while larger and heavier good seed stays at the bottom. Separations are accurately controlled by a calibrated valve cap at the top of the column. The filled seeds were then stored in paper bags at 25 °C with a relative humidity of less than 50% until seed testing. SY was recorded for each plot. SMC, PP, TSW, and GP were assessed using the test methods of the ISTA for *U. ruziziensis* (ISTA, 2011). TSW was measured using the weight of a pure-seed spikelet. Seed purity testing was performed by separating the pure seed fraction from inert materials and other seeds, and then separately weighing the pure seed fraction. The PP was calculated by multiplying the weight of pure seed by 100 and dividing it by the total weight of the sample. Germination tests were carried out 90 days after the seeds were harvested. SY was adjusted to 11% SMC. TN, IN, FTN/P, FTP, and FSP were then calculated. FTN × 100/TN was used to determine the FTP, while PSY was calculated as SY × PP/100, and PGSY as PSY × GP/100.

Statistical Analysis

The ANOVA was performed using a split-plot design in RCBD with the four main-plot treatments and four sub-plot treatments replicated four times for yield and quality properties. Significant differences among the treatment means were tested by the LSD with the R software version 4.1.0 (R Core Team, 2021). The Pearson's correlations were calculated for the CCD and N-rate with seed yield, seed yield components, and seed quality, and significant differences were declared among the means when $p < 0.05$.

Figure 2-3 Grasses were cut by hand at the time specified for each closing cut date.

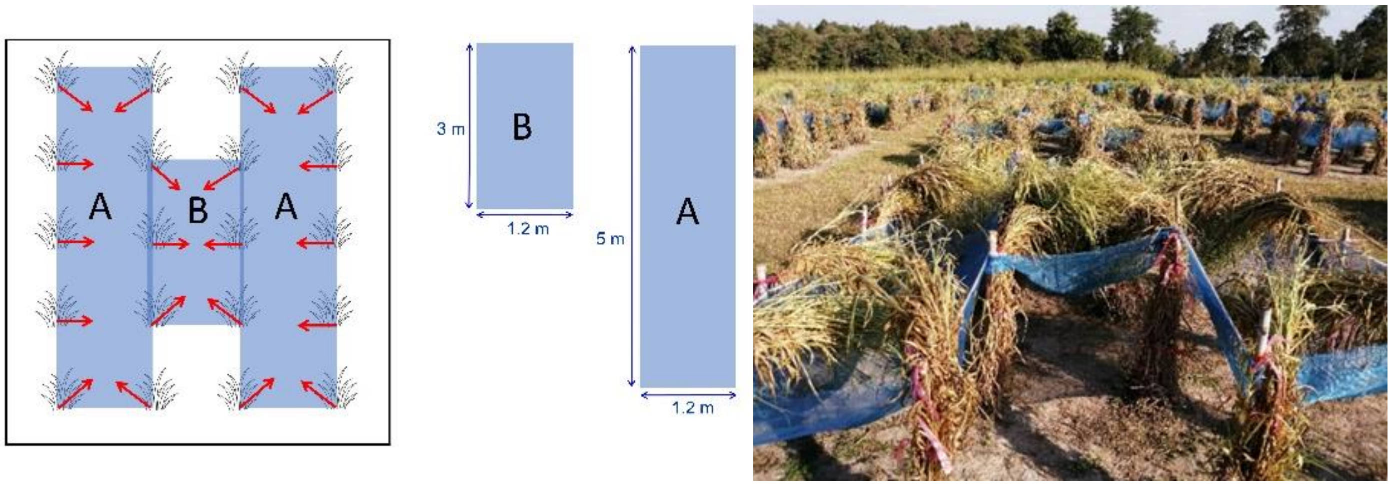

Figure 2-4 Setting three nylon net sheets in the plot.

Figure 2-5 Experimental plots during seed harvesting.

Results and Discussion

Soil and Weather Conditions

The chemical properties of preliminary soils at the experimental sites are shown in Table 2-11. The soils at both experimental sites were acidic with low OM content (< 1%) and were deficient in essential minerals. The cultivars 'OKI-1' and 'Br-203' demonstrated edaphic adaptation in terms of growth and productivity (LDD, 2010). Our findings were confirmed by Rao et al. (1996), who reported that most commercial *Urochloa* spp. are adaptable to a wide range of soil types; from poor, acidic soils to highly fertile, neutral soils.

In soil samples collected immediately after seed harvest, the pH, phosphorus, and potassium levels were lower than before sowing, whereas the amounts of OM and N were slightly higher. The element values in each treatment were similar (Tables 2-12 and 2-13).

Increasing seed production in *Urochloa* spp. mostly relies on their adaptability to the climate. Seed set and pure live seed yield were substantially affected by weather conditions, particularly rainfall in the pre-heading stage (Boonman, 1973). *Urochloa* spp. are typically grown in areas with higher annual rainfall (> 2000 mm/year), but they have been grown in areas with a minimum annual rainfall of roughly 1000 mm in Australia (Stür et al., 1996), and in some *Urochloa* hybrids, even in places with an annual rainfall as low as 700 mm and a dry season of 5–6 months. During this study period (7 December 2018 to 24 December 2019), the annual rainfall was 855.9 mm with 96 rainy days, conforming to the range mentioned above (Figure 2-1(A)). The grasses received 112.47 mm/month of rain in the growing period (June to November 2019), which is considered sufficient for growth. Similarly, Bouathong et al. (2011) reported that *Urochloa* grass requires 83–125 mm of rainfall each month during its growth period. In this study, the maximum rainfall occurred in August 2019 (330.1 mm with 24 rainy days). During that time, the grasses were regrowing after closed cutting and applying N, which meant that the timing of rainfall was well suited for the growth of the *Urochloa* cultivars.

It is well known that the optimal temperature and precipitation are necessary to increase pasture grass seed yield and quality (Nadew, 2018). In our trial, the monthly average relative humidity, the monthly average minimum, and the maximum temperature were shown in Figure 2-1(B). During the experimental period, the average temperature and relative humidity in 2018 and 2019 were 26.6 ± 1.9 °C, 27.3 ± 3.0 °C, and 79.5 ± 8.3%, 71.6 ± 12.6%, respectively. Bouathong et al. (2011) reported that the average temperature during their experiment was 27.3 °C, which was within the optimum range of 25–35 °C for *Urochloa* species. This range was similar to that reported by Wongsuwan (1994) (in (Deinum & Dirven 1972)) for the optimum day- and night-time temperatures for *U. ruziziensis*, which were 33 °C and 28 °C, respectively. From these reports, the average temperature at our study time is suitable for seed production of the two cultivars of *Urochloa* spp.

Figure 2-1(C) depicts the monthly average day length and average daily sunshine duration at the experimental sites. At the experimental sites, the average day length during the trial period was approximately 12 h/day, and the average sunshine duration was 6 h/day, with the shortest duration in August (3 h/day) and the longest duration in April (9 h/day). These conditions were most likely sufficient for *Urochloa* cultivars to grow and flower. This corroborates the findings of Wongsuwan (1994), who reported that day length is crucial for triggering the reproductive development of *U. ruziziensis* in Thailand. They also found that the highest ruzi seed yields were obtained at 11-h day lengths compared with the longer day lengths (14, 13 and 12 h).

Effects of CCD and N-rate on Seed Yield, Seed Yield Components, and Seed Quality in the Two Novel Cultivars of Urochloa spp.

In 2019, the period of cv. 'OKI-1' growth following the CCD to the beginning of the seed harvest in the treatments that were cut on 15 June, 1 July, and 15 July was 163 days, 148 days, and 133 days, respectively; the period of growth in the uncut treatment was 354 days.

For cv. 'Br-203', the growth period from the beginning of each CCD till the beginning of harvest was longer than in cv. 'OKI-1' for three days: 166 days, 151 days, and 136 days for the CCD on 15 June, 1 July, and 15 July, respectively, and it was 357 days for the uncut treatment. Onset of flowering in both cultivars occurred at approximately the same time (13–29 October). The duration of seed harvesting was similar among all treatments in each cultivar (cv. 'OKI-1'; 25 November–24 December and cv. 'Br-203'; 28 November–28 December).

In both trials, the analysis of variance showed that there were no significant interactions between CCD and N-rate for any of the traits measured, which is consistent with the findings of Nakmanee et al. (2007b) on cv. 'Mulato' in Northeast Thailand. Lodging was not observed in any of the treatments because all plants were tied to a bamboo stake inserted into the ground. Therefore, tiller death and seed shedding were not caused by lodging.

In both trials, several parameters, including seed yields, were not influenced by N-rate. This might be because of the incomplete N uptake and excessive rainfall after applying N fertilizer, which can cause soluble nitrate ions to be leached out of the rooting zone, diminishing N use efficiency (Galdos et al., 2020). N top-dressed as urea is expected to be lost through leaching and volatilization. Additionally, high temperatures also cause higher rates of NH_3 volatilization because they increase the concentrations of NH_3 dissolved in soil water (Jones et al., 2007). Furthermore, because the soil at the experimental sites is a single-grain structure, N loss into the ground is possible. This is supported by the earlier studies (Jones et al., 2007; Sun et al., 2008), which reported that various soil and climate factors interact to affect urea volatilization, and N responses are also affected by climate, geographical factors, and soil factors, such as type, texture, drainage, pH, fertility, moisture, and temperature. However, the efficiency of N application varies with plants, soil, seasons, climatic conditions, and application techniques. By understanding soil and climate factors that influence volatilization, avoiding applying urea in situations that may enhance volatilization or adopting best management practices to minimize the risk of loss is needed. Another possible reason for this is that when low soil fertility is caused by a deficiency of other nutrients rather than N, responses to N will be improved once these nutrients are present in sufficient amounts (Sun et al., 2008). Further study is required to evaluate the timing of N fertilizer application on seed yield, efficiency of N use, and apparent N efficiency of cv. 'OKI-1' and cv. 'Br-203'.

N could be applied to seedlings during the vegetative to spikelet initiation period. Adequate N from floral initiation to seedhead emergence can boost seed yield by improving tiller survival, increasing seedhead density, seedhead branching, floret differentiation, and seed size (Gbenou et al., 2018; Brunse & Watkin, 1992). However, in our study, the N fertilizer was applied once in one dose after the closing cut immediately. This means plants do not get efficient fertilizer until the reproductive growth period. We have found that many tillers become yellowish from the start of seed harvest until the end of harvest. Furthermore, previous studies have shown that a split N application can improve nutrient use efficiency, promote optimal seed yields, and reduce nutrient loss (Catuchi et al., 2019; Faji Dida et al., 2021). Therefore, to achieve high seed yields in the two novel *Urochloa* cultivars, besides N fertilizer application after closing cut, further application of N before flowering may be required.

Trial 1: Effect of CCD and N-rate on Seed Yield, Seed Yield Components, and Seed Quality in Cultivar 'OKI-1'

As shown in Table 2-14, the CCD had a significant effect on seed yield and some seed yield components. The highest seed yield was obtained from the 1 July CCD, while the lowest seed yields were obtained from the uncut treatment and the 15 July CCD. Canto et al. (2016) reported that crops with later CCD treatments and lower N nutrition rates, which caused the low leaf area, had a reduced ability to provide carbohydrates for seed production, along with a short period for the commencement of spikelets in new tillers. The results are consistent with an earlier study on CCD in cv. 'Mulato' grown in Thailand; the lowest PSY was obtained from the uncut plots and the delayed CCD (15 August) (Nakmanee et al., 2007b). A study on *Megathyrsus maximus* cv. 'Mombaza' grown in Mexico by Joaquín et al. (2020) also confirmed that the closing cut (precut) has a positive effect on the yield and quality of the guineagrass seed, with the highest yield being found with the CCD of July 20 when compared with the treatment without closing cut and the other delayed CCD treatments. However, this finding contrasted with a study on *U. ruziziensis* by Phaikaew et al. (1985) in which the highest PSY was obtained in uncut plots. Similarly, Satjipanon et al. (1987) showed that the highest PSY in the second year of the experiment was also obtained in uncut plots. As hypothesized, our experiments prove that the CCD was found to be particularly critical for seed production. This supports previous studies on *Urochloa* hybrids conducted in Thailand (Hare et al., 2007b). The proper time to implement a closing cut on the seed crops of cv. 'OKI-1' was 147–163 days (15 June–1 July) before seed harvest.

The total number of tillers, the percentage of tillers that went through the reproductive stage, and the number of reproductive tillers are the variables that are most highly correlated with seed production (Lopes et al., 2016). Several studies (e.g., Boonman, 1971; Humphreys & Riveros, 1986; Hill & Loch, 1993) have reported that optimum tiller density affects increased forage and seed yields. The poor seed yield of the uncut crop could be attributed to intra-plant competition because many tillers remained vegetative despite the otherwise favorable conditions for reproductive development. The closing cut increases seed yields by removing the shading effect of cumulative forage plants and allowing sunlight to penetrate the grass canopy, resulting in more rapid tiller emergence (Brunse & Watkin, 1992). In addition, the closing cut is favorable to the competition for the reserves and photoassimilates in the leaves, stems, and young inflorescences of the emerged tillers, which affect the final mass of seed. The competition is influenced by the tiller hierarchy, i.e., tillers of smaller orders form heavier seeds since they receive more assimilates (Hare et al., 2007b; Martiniello & Silva, 2011; Awad et al., 2013). If plants are left uncut, they will grow to be overly tall, resulting in a decreased seed yield (Gobius et al., 1998). However, not much information has been specifically published on *Urochloa* grass with regard to tiller density and seed yield, or on the interrelationship between these two traits.

The closing cut on 15 June significantly increased TN and SN/R ($p < 0.05$); however, IN/T was the lowest for this treatment. The 1 July CCD produced the highest FTP and IN/T, but SN/R was the lowest, the same as the 15 July CCD.

The low FTN and IN values found for the 15 July CCD were caused by the low fertility of late-emerging tillers. In addition, the 15 July closing cut decreases seed yields because many FTN are removed. The removal of many potential tillers reduces the number of inflorescences and results in inflorescences with fewer spikelets, even in the research carried out in *Lolium* spp. (FAR, 2016). These findings clearly show that FTP and IN had a positive relationship (Table 2-15). This corroborates with previous results (Lopes et al., 2016), which reported that late cuts in relation to the cycle of forage grasses can have negative effects on the productivity of seeds, possibly by reducing the photosynthetic area and eliminating reproductive parts of the tiller. The increased IN in the closing cut treatments was found to be related to the increased seed yields. The study on *Pennisetum* spp. and *Andropogon* spp. by Mishra & Chatterjee (1968) reported that a decrease in tiller fertility due to a delayed closing cut was strongly related to reduced seed yields, and it was quite possible that the apices of many of the early-forming tillers were destroyed. Additionally, a significantly lower SN/R was observed for the 1 July and 15 July CCDs in this trial. These findings were similar to those of Wongsuwan (1999), who reported that the late closing cut severely depressed both floret numbers and fertilized seed numbers in *U. ruziziensis*.

The seed quality of cv. 'OKI-1' was also affected by the CCD. The highest FSP, TSW, and PP were obtained from the 1 July closing cut (Table 2-16). The lowest TSW was observed when the closing cut was delayed until 15 July. The uncut treatment had significantly reduced FSP and PP values ($p < 0.05$) when compared with the 1 July treatment. The obviously low PP and GP values were observed in the uncut and 15 July CCD treatments when compared with the 15 June and 1 July CCDs. These low values also led to low PSY and PGSY significantly ($p < 0.05$). Although a significant difference was observed in PP, seed purity was very high in each treatment due to good management from seed setting until seed harvesting and cleaning. The most marked effect of the CCD on these seed quality attributes in OKI-1 was gained from the CCD on 1 July, as shown in Table 2-16. In this trial, the 15 July CCD had a lower TSW than the other CCD treatments. This tendency was similar to that observed in seed quality in *P. atratum* in Thailand in that a May–July CCD had a higher TSW than an August CCD (Hare et al., 1999), and similar to cv. 'Mulato' in Thailand (Nakmanee et al., 2007b), where TSW decreased following the delayed CCD (comparing between 15 June, 15 July and 15 August). This was due to the duration of growth after cutting until flowering being shorter than it was in the other treatments, which caused a decrease in seed size (Humphreys & Riveros, 1986).

The CCD was not significantly correlated with all the seed quality attributes in cv. 'OKI-1' ($p > 0.05$) (Table 2-17). The reasons for the low PSY and PGSY were related to the uncut treatment's having a low FSP and a low PSY, because the FSP was positively correlated with PSY and PGSY (Table 2-17).

The N-rate had no significant effect on the seed yields in cv. 'OKI-1' (Table 2-14). However, the N-rate had a significant ($p < 0.05$) effect on seed yield components; the applied N enhanced TN and increasing N-rates decreased FTP. On the other hand, the lowest RN/I value was observed at the highest N-rate (150 kg N/ha). This is in contradiction with earlier findings (Loch et al., 1999a; Kumar et al., 2005), which reported that the positive response of tropical and subtropical grasses to N fertilizer was primarily due to an increase in the number of fertile tillers, increased spike length, and an increase in the number of spikelets/spikes.

The N-rate of 150 kg N/ha produced approximately 16% more TN than the control treatment. Our results were similar to those obtained in previous studies on *U. decumbens* and *U. ruziziensis*, which showed that TN increased with an increase in the N-rate (Benteo et al., 2016; Canto et al., 2020). Wongsuwan (1999) also found that the addition of 150 and 250 kg N/ha significantly increased total reproductive tiller numbers. In our study, the application of 150 kg N/ha to cv. 'OKI-1' decreased FTP and RN/I by approximately 13% and 10%, respectively, compared with the control treatment. The strong and significant negative correlation between N-rate and FTP was found only in cv. 'OKI-1' (Table 2-18). As reported by Langer (1980), the number of fertile tillers is not necessarily related to increased N application levels, likely because of unfavorable partitioning of assimilates between leaves for growth and seed production, greater competition for assimilates in a lodged crop, and possibly due to inadequate pollination.

Additionally, N application significantly decreased the seed quality attributes, such as FSP, TSW, and PP ($p < 0.05$) (Table 2-16). According to our findings, increasing the N-rate gradually decreased FSP and TSW in cv. 'OKI-1'**.** A negative correlation was observed between seed quality and N-rate in cv. 'OKI-1', as shown in Table 2-19.

Trial 2: Effect of CCD and N-Rate on Seed Yield, Seed Yield Components and Seed Quality in Cultivar 'Br-203'

The effect of CCD on seed yield and seed yield components was not significant ($p > 0.05$), except for SN/R as shown in Table 2-20. The results could be explained by the fact that all tussocks of grass were supported with bamboo stakes and tied the inflorescences to the stakes with plastic coated wire, resulting in no tiller damage and no seed loss due to lodging. Conversely, in several previous studies (Hare et al., 1999; Phaikaew et al., 2002), the lower seed yields in earlier closed crops were observed to be caused by severe lodging at seed harvest. The uncut treatment had the highest SN/R, while the lowest SN/R was shown in the delayed closing cut (15 July). Moreover, a delay in the CCD likely caused a decrease in TN, FTN, FTP, and RN/I. This supports previous findings in *P. atratum* grown in Thailand (Hare et al., 1999), which reported that inflorescences produced from plants cut in August (delayed closing cut) had fewer racemes per inflorescence and generally fewer spikelets per raceme compared with inflorescences from plants cut earlier. Since the last CCD of the study was on 15 July, we were unable to clearly indicate the relationships between the delayed CCD and the seed yields. However, the CCD treatments applied in this study corresponds to the recommended closing cut period for cv. 'Mulato' and cv. 'Mulato II' seed crops in Thailand (early July–early August) (Hare et al., 2007b) as well as the recommendation of CCD for *U. ruziziensis* (Phaikaew et al. 1985) and *P. atratum* (Hare et al., 1999; Phaikaew et al., 2002) should also be performed in early July–early August.

A significant correlation ($p < 0.05$) between CCD and FTP in the cv. 'Br-203' is shown in Table 2-21. TSY has a positive correlation with IN and IN/T while having a negative correlation with other seed components. CCD had no effect on seed quality attributes (Table 2-22). However, CCD had a significant correlation with FSP in cv. 'Br-203' (Table 2-23).

In the findings in several tropical pasture grasses, N fertilizer application to seed crops affects yield component dynamics such as tiller increase, number of fertile tillers, florets per spikelet, and seed per head, resulting in higher seed yield and quality (Gbenou et al., 2018). However, in this experiment, all the measured agronomic traits, except TSW, were not significantly *($p > 0.05$)* affected by the variation in N-rate. Average seed yield, seed yield components, and seed quality attributes of cv. 'Br-203' in response to different N-rates are presented in Tables 2-20 and 2-22. The addition of N tends to increase IN and IN/T in cv. 'Br-203' (Table 2-20), though no significant difference was observed. This is consistent with a strong positive correlation that was observed between TN, IN, and IN/T and the N-rate (Table 2-24).

The effect of N on seed quality varies depending on the species and environmental conditions (Gbenou et al., 2018). A negative correlation was observed between seed quality and N-rate in cv. 'Br-203', as shown in Table 2-25. According to our findings, higher levels of N-rate gradually decreased TSW in cv. 'Br-203'. Even though these results differ from previous studies on *U. decumbens* (Canto et al., 2020)*, U. ruziziensis* (Wongsuwan, 1999), and cv. 'Mulato' (Nakmanee et al., 2007b), they are consistent with those of earlier studies in *P. atratum* at Pakchong, Thailand (Phaikaew et al., 2002). For example, in hybrid *Urochloa* lines in which an N-rate of 80 kg N/ha was applied, TSW was reduced when compared with an N-rate of 40 kg N/ha (Bouathong et al., 2011). Meijer & Vreeke (1988) reported that average seed weight was reduced at high N-rates because the increase in the proportion of late-developing inflorescences results in lighter seed weight at harvest.

According to Nakmanee et al. (2015), cv. 'Br-203' has adapted well to the conditions in northeastern and northern Thailand, and this cultivar has high agronomic potential, including the ability to tolerate drought conditions. The cultivar appeared to have enough inflorescences, racemes, and spikelets, indicating a potential for favorable seed yields. However, this cultivar also produced numerous empty seeds, which decreased seed yields and the low seed-set observed was a crucial weakness of the cultivar as well as hybrid *Urochloa* grasses, and a poor seed-set could be a common flaw in newly formed apomictic forage grass hybrids (Hare et al., 2015a; Miles & Hare, 2007).

An early study on cv. 'Mulato II' reported very low seed yields with fewer than 2% of the spikelets that formed viable seed, likely due to pollen sterility (Hare et al., 2007b). One reason for low seed setting is thus partly genetic in origin. For example, Risso-Pascotto et al. (2004; 2005) reported that more than 65% of pollen grains in interspecific hybrids between *U. ruziziensis* and *U. brizantha* were sterile. Specifically, they demonstrated that this sterility had a genetic basis and was due to irregular chromosome segregation, chromosome stickiness, abnormal cytokinesis, and asynchronous meiosis.

In both trials, close cutting is required for seed production in both *Urochloa* cultivars. Mid-June to early July was the best period for close cutting cv. 'OKI-1', and early-July to mid-July was the proper time for close cutting cv. 'Br-203'. This study discovered that N fertilizer application had no direct effect on seed yield. This is supported by the study on *Cenchrus* spp., which reported that the amount of N fertilizer required depends on species, soil type, and rainfall (Gbenou et al., 2018). The low soil fertility, which is caused by a deficiency of other nutrients besides N, affected the plants' responses to N. Once these nutrients are present in sufficient concentrations, the N response may improve (Sun et al., 2008). More often, 100–250 kg N/ha and a rate of more than 300 kg N/ha caused a reduction in seed yield due to severe plant lodging (Gbenou et al., 2018).

Moreover, N fertilization management can affect the seed yield and quality of tropical forage grasses. The responses of tropical grasses as a function of N fertilization management may vary between species and cultivars (Catuchi et al., 2019). Additionally, in terms of economic performance and the relationship between seed production and N-rate, the results of this study showed that no N fertilizer was appropriate for the two novel *Urochloa* cultivars in northern Thailand. However, further studies should be conducted to confirm these results.

Table 2-11 Chemical properties of preliminary soils collected from both experimental sites

Trial	pH	OM (%)	Total N (%)	Extractable (mg/kg)										EC (dS/m)
				P	K	Ca	Mg	Na	S	Mn	Fe	Cu	Zn	
1	5.39	0.67	0.03	0.89	39.84	614.38	36.66	12.44	3.03	14.15	55.70	0.24	0.07	0.83
2	5.68	0.73	0.04	0.87	85.23	225.66	23.10	9.03	2.43	11.76	26.45	0.02	0.04	0.70

Abbreviations: EC, electric conductivity; OM, organic matter.

Table 2-12 Chemical properties of post-harvest soils in the experimental field in Trial 1

N-rate (kg N/ha)	pH	OM (%)	Total N (%)	Extractable (mg/kg)	
				P	K
0	5.15	1.53	0.08	0.71	24.74
50	4.76	0.94	0.05	0.86	25.24
100	5.16	0.85	0.04	0.67	27.99
150	5.05	1.24	0.06	0.68	25.76

Table 2-13 Chemical properties of post-harvest soils in the experimental field in Trial 2

N-rate (kg N/ha)	pH	OM (%)	Total N (%)	Extractable (mg/kg)	
				P	K
0	5.12	1.16	0.06	0.71	39.15
50	5.23	1.23	0.06	0.89	44.04
100	5.15	1.19	0.06	0.84	45.46
150	5.18	1.16	0.06	0.82	34.52

Table 2-14 Effect of CCD and N-rate on seed yield and seed yield components in cultivar 'OKI-1'

Treatment	**TSY (kg/ha)**	**TN (no./m^2)**	**FTN (no./m^2)**	**IN (no./m^2)**	**FTP (%)**	**IN/T (no.)**	**RN/I (no.)**	**SN/R (no.)**
CCD								
Uncut	484.96^{b}	94.62^{b}	83.31	173.60	88.37^{ab}	2.07^{ab}	3.54	37.05^{ab}
15 June	510.23^{ab}	105.25^{a}	89.31	171.67	86.30^{ab}	1.87^{b}	3.26	38.34^{a}
1 July	660.44^{a}	92.88^{b}	85.90	182.65	92.81^{a}	2.14^{a}	3.61	34.79^{b}
15 July	479.29^{b}	99.69^{ab}	80.85	157.39	82.01^{b}	1.90^{ab}	3.61	34.58^{b}
CV (%)	40.20	12.20	19.70	25.50	13.10	16.70	14.50	12.30
N-rate (kg N/ha)								
0	587.25	87.42^{b}	80.96	174.03	93.01^{a}	2.13	3.73^{a}	36.05
50	562.57	100.94^{a}	89.04	175.19	88.45^{ab}	1.95	3.40^{ab}	35.24
100	443.62	100.15^{a}	86.38	159.54	86.62^{ab}	1.85	3.66^{a}	37.01
150	541.47	103.94^{a}	83.00	176.54	81.41^{b}	2.05	3.23^{b}	36.44
CV (%)	45.70	14.90	23.20	31.50	17.20	21.00	15.20	12.20
Mean	533.73	98.11	84.84	171.33	87.37	2.00	3.51	36.19
CCD × N-rate	ns	ns	ns	ns	ns	ns	ns	ns

Abbreviations: TSY, total seed yield; TN, tiller number per square meter; FTN, fertile tiller number per square meter; IN, inflorescence number per square meter; FTP, fertile tiller percentage; IN/T, inflorescence number per tiller; RN/I, raceme number per inflorescence; SN/R, spikelet number per raceme; ns, non-significant. Note: Values followed by different superscript letters indicate statistical differences within each column for CCD and N-rate ($p < 0.05$).

Table 2-15 Correlation coefficients for seed yield, seed components and CCD in cultivar ‘OKI-1’

Traits	**CCD**	**TSY**	**TN**	**FTN**	**IN**	**FTP**	**IN/T**	**RN/I**
TSY	−0.3600							
TN	−0.3668	−0.5223						
FTN	−0.1675	0.3454	0.4199					
IN	0.2100	0.7733	−0.4966	0.5782				
FTP	0.1931	0.8311	−0.6407	0.4271	0.9817 **			
IN/T	0.3686	0.6802	−0.9443 *	−0.0990	0.7543	0.8555		
RN/I	0.0378	0.2722	−0.8100	−0.8042	−0.0536	0.1361	0.5890	
SN/R	0.4129	−0.3906	0.5640	0.6699	0.1631	−0.0174	−0.3566	−0.8943 *

Abbreviations: CCD, closing cut date; TSY, total seed yield; TN, tiller number per square meter; FTN, fertile tiller number per square meter; IN, inflorescence number per square meter; FTP, fertile tiller percentage; IN/T, inflorescence number per tiller; RN/I, raceme number per inflorescence; SN/R, spikelet number per raceme. Note: Values without asterisks are not significant, * correlation is significant at the 0.05 level (two-tailed), $p < 0.05 = 0.8783$ and ** correlation is significant at the 0.01 level (two-tailed), $p < 0.01 = 0.9587$.

Table 2-16 Effect of CCD and N-rate on seed quality in cultivar 'OKI-1'

Treatment	FSP (%)	TSW (g)	PP (%)	PSY (kg/ha)	GP (%)	PGSY (kg/ha)
CCD						
Uncut	24.56^{b}	8.42^{ab}	98.73^{b}	478.82^{b}	91.62	439.71^{b}
15 June	28.55^{ab}	8.51^{ab}	99.00^{ab}	504.87^{ab}	93.02	474.05^{ab}
1 July	35.08^{a}	8.58^{a}	99.22^{a}	654.97^{a}	93.28	611.31^{a}
15 July	28.70^{ab}	8.32^{b}	98.72^{b}	473.39^{b}	91.38	432.55^{b}
CV (%)	28.40	3.40	0.50	40.40	3.50	41.40
N-rate (kg N/ha)						
0	33.52^{a}	8.72^{a}	99.30^{a}	582.89	92.55	539.26
50	30.03^{ab}	8.49^{ab}	98.47^{b}	553.68	92.97	514.97
100	27.52^{b}	8.34^{b}	98.99^{ab}	439.62	92.31	408.71
150	25.82^{b}	8.29^{b}	98.91^{ab}	535.86	91.47	494.68
CV (%)	21.50	4.30	0.80	45.50	5.10	46.30
Mean	29.22	8.46	98.92	528.01	92.32	489.40
CCD × N-rate	ns	ns	ns	ns	ns	ns

Abbreviations: FSP, filled seed percentage; TSW, thousand seed weight; PP, purity percentage; PSY, pure seed yield; GP, germination percentage; PGSY, pure germinated seed yield; ns, non-significant. Note: Values followed by different superscript letters indicate statistical differences within each column for CCD and N-rate ($p < 0.05$).

Table 2-17 Correlation coefficients for seed quality and CCD in cultivar 'OKI-1'

Traits	**CCD**	**FSP**	**TSW**	**PP**	**PSY**	**GP**
FSP	−0.7112					
TSW	−0.1391	0.6318				
PP	−0.4614	0.8609	0.9326 *			
PSY	−0.3621	0.9089*	0.8176	0.9161 *		
GP	−0.4023	0.7084	0.9515 *	0.9596 **	0.7725	
PGSY	−0.3711	0.9072 *	0.8460	0.9376*	0.9983 **	0.8082

Abbreviations: CCD, closing cut date; FSP, filled seed percentage; TSW, thousand seed weight; PP, purity percentage; PSY, pure seed yield; GP, germination percentage; PGSY, pure germinated seed yield. Note: Values without asterisks are not significant, * correlation is significant at the 0.05 level (two-tailed), $p < 0.05 = 0.8783$ and ** correlation is significant at the 0.01 level (two-tailed), $p < 0.01 = 0.9587$.

Table 2-18 Correlation coefficients for seed yield, seed components and N-rate in cultivar ‘OKI-1’

Traits	**N-rate**	**TSY**	**TN**	**FTN**	**IN**	**FTP**	**IN/T**	**RN/I**
TSY	−0.5259							
TN	0.8610	−0.4558						
FTN	0.1248	−0.3545	0.5828					
IN	−0.1323	0.9084 *	−0.0668	−0.2586				
FTP	−0.9856 **	0.3914	−0.8884 *	−0.1462	−0.0247			
IN/T	−0.3612	0.8466	−0.5798	−0.7948	0.7571	0.2823		
RN/I	−0.6904	−0.1618	−0.7842	−0.2286	−0.5548	0.8025	−0.0296	
SN/R	0.5155	−0.7649	0.1253	−0.3261	−0.7035	−0.3715	−0.3048	0.2403

Abbreviations: N-rate, rate of nitrogen application; CCD, closing cut date; TSY, total seed yield; TN, tiller number per square meter; FTN, fertile tiller number per square meter; IN, inflorescence number per square meter; FTP, fertile tiller percentage; IN/T, inflorescence number per tiller; RN/I, raceme number per inflorescence; SN/R, spikelet number per raceme. Note: Values without asterisks are not significant, * correlation is significant at the 0.05 level (two-tailed), $p < 0.05 = 0.8783$ and ** correlation is significant at the 0.01 level (two-tailed), $p < 0.01 = 0.9587$.

Table 2-19 Correlation coefficients for seed quality and N-rate in cultivar 'OKI-1'

Traits	**N-rate**	**FSP**	**TSW**	**PP**	**PSY**	**GP**
FSP	−0.9880 **					
TSW	−0.9630 **	0.9929 **				
PP	−0.2450	0.3445	0.3963			
PSY	−0.5310	0.6107	0.6767	0.0477		
GP	−0.7968	0.6971	0.6148	−0.3038	0.2000	
PGSY	−0.5455	0.6214	0.6845	0.0298	0.9996 **	0.2267

Abbreviations: N-rate, rate of nitrogen application; FSP, filled seed percentage; TSW, thousand seed weight; PP, purity percentage; PSY, pure seed yield; GP, germination percentage; PGSY, pure germinated seed yield. Note: Values without asterisks are not significant, ** correlation is significant at the 0.01 level (two-tailed), $p < 0.01 = 0.9587$.

Table 2-20 Effect of CCD and N-rate on seed yield and seed yield components in cultivar 'Br-203'

Treatment	TSY (kg/ha)	TN (no./m^2)	FTN (no./m^2)	IN (no./m^2)	FTP (%)	IN/T (no.)	RN/I (no.)	SN/R (no.)
CCD								
Uncut	303.17	163.60	127.46	315.00	76.86	2.30	3.42	36.33^{a}
15 June	298.85	173.81	126.25	293.93	73.73	2.28	3.39	34.62^{ab}
1 July	339.76	163.12	119.58	308.38	71.93	2.29	3.31	34.45^{ab}
15 July	361.02	162.75	115.42	327.30	69.56	2.36	3.05	30.77^{b}
CV (%)	45.30	12.80	18.80	32.20	21.40	19.30	21.50	14.60
N-rate (kg N/ha)								
0	411.91	158.92	120.12	293.32	75.83	2.17	3.29	32.21
50	387.20	161.73	118.71	293.64	72.16	2.27	3.07	35.38
100	302.22	167.85	114.58	310.15	67.00	2.38	3.30	32.83
150	374.70	174.79	135.29	347.50	77.09	2.41	3.51	35.75
CV (%)	63.90	17.50	42.70	69.40	36.80	49.20	23.90	16.40
Mean	325.70	165.82	122.18	311.15	73.02	2.31	3.29	34.04
CCD × N-rate	ns	ns	ns	ns	ns	ns	ns	ns

Abbreviations: TSY, total seed yield; TN, tiller number per square meter; FTN, fertile tiller number per square meter; IN, inflorescence number per square meter; FTP, fertile tiller percentage; IN/T, inflorescence number per tiller; RN/I, raceme number per inflorescence; SN/R, spikelet number per raceme; ns, non-significant. Note: Values followed by different superscript letters indicate statistical differences within each column for CCD ($p < 0.05$).

Table 2-21 Correlation coefficients for seed yield, seed components and CCD in cultivar 'Br-203'

Traits	**CCD**	**TSY**	**TN**	**FTN**	**IN**	**FTP**	**IN/T**	**RN/I**
TSY	−0.6000							
TN	−0.1754	−0.6503						
FTN	0.7072	−0.9893 **	0.5337					
IN	0.0675	0.7285	−0.8455	−0.6401				
FTP	0.8914 *	−0.8821 *	0.2174	0.9413 *	−0.3919			
IN/T	−0.2454	0.7815	−0.5508	−0.7522	0.8946 *	−0.6356		
RN/I	0.5993	−0.9167 *	0.4430	0.9289 *	−0.7345	0.8879 *	−0.9195 *	
SN/R	0.7261	−0.8437	0.2263	0.8892*	−0.5645	0.9288 *	−0.8398	0.9724

Abbreviations: CCD, closing cut date; TSY, total seed yield; TN, tiller number per square meter; FTN, fertile tiller number per square meter; IN, inflorescence number per square meter; FTP, fertile tiller percentage; IN/T, inflorescence number per tiller; RN/I, raceme number per inflorescence; SN/R, spikelet number per raceme. Note: Values without asterisks are not significant, * correlation is significant at the 0.05 level (two-tailed), $p < 0.05 = 0.8783$ and ** correlation is significant at the 0.01 level (two-tailed), $p < 0.01 = 0.9587$.

Table 2-22 Effect of CCD and N-rate on seed quality in cultivar 'Br-203'

Treatment	FSP (%)	TSW (g)	PP (%)	PSY (kg/ha)	GP (%)	PGSY (kg/ha)
CCD						
Uncut	18.25	5.72	95.93	290.90	93.03	269.56
15 June	12.38	5.79	95.21	287.54	91.72	264.05
1 July	14.61	5.72	95.31	325.12	91.06	299.10
15 July	12.12	5.90	87.68	340.75	93.17	318.26
CV (%)	61.90	6.60	13.60	46.80	6.30	48.30
N-rate (kg N/ha)						
0	16.40	5.90^{ab}	90.08	301.62	93.90	282.18
50	12.17	5.98^{a}	97.36	331.58	92.28	304.43
100	14.43	5.73^{ab}	92.62	289.13	90.72	266.32
150	14.33	5.55^{b}	94.07	321.96	92.12	298.04
CV (%)	64.50	8.60	13.60	64.80	5.50	65.20
Mean	14.37	5.78	93.53	311.07	92.23	287.74
CCD × N-rate	ns	ns	ns	ns	ns	ns

Abbreviations: FSP, filled seed percentage; TSW, thousand seed weight; PP, purity percentage; PSY, pure seed yield; GP, germination percentage; PGSY, pure germinated seed yield; ns, non-significant. Note: Values followed by different superscript letters indicate statistical differences within each column for N-rate ($p < 0.05$).

Table 2-23 Correlation coefficients for seed quality and CCD in cultivar ‘Br-203’

Traits	CCD	FSP	TSW	PP	PSY	GP
FSP	0.9171 *					
TSW	−0.5491	−0.7422				
PP	0.4982	0.5872	−0.9377 *			
PSY	−0.6109	−0.4277	0.5556	−0.7727		
GP	0.4388	0.1978	0.4964	−0.5415	0.0913	
PGSY	−0.5743	−0.4116	0.5895	−0.8070	0.9972 **	0.1649

Abbreviations: CCD, closing cut date; FSP, filled seed percentage; TSW, thousand seed weight; PP, purity percentage; PSY, pure seed yield; GP, germination percentage; PGSY, pure germinated seed yield. Note: Values without asterisks are not significant, * correlation is significant at the 0.05 level (two-tailed), $p < 0.05 = 0.8783$ and ** correlation is significant at the 0.01 level (two-tailed), $p < 0.01 = 0.9587$.

Table 2-24 Correlation coefficients of seed yield, seed components and N-rate in cultivar ‘Br-203’

Traits	N-rate	TSY	TN	FTN	IN	FTP	IN/T	RN/I
TSY	−0.5385							
TN	0.9845 **	−0.4567						
FTN	0.5900	0.3364	0.6823					
IN	0.9074 *	−0.2248	0.9633 **	0.8406				
FTP	−0.0394	0.8285	0.0880	0.7835	0.3438			
IN/T	0.9771 **	−0.6991	0.9357 *	0.4050	0.8068	−0.2508		
RN/I	0.6394	−0.1345	0.7618	0.7318	0.8621	0.4199	0.5333	
SN/R	0.5845	0.1445	0.5316	0.6348	0.5324	0.3285	0.4927	0.0721

Abbreviations: N-rate, rate of nitrogen application; TSY, total seed yield; TN, tiller number per square meter; FTN, fertile tiller number per square meter; IN, inflorescence number per square meter; FTP, fertile tiller percentage; IN/T, inflorescence number per tiller; RN/I, raceme number per inflorescence; SN/R, spikelet number per raceme. Note: Values without asterisks are not significant, * correlation is significant at the 0.05 level (two-tailed), $p < 0.05 = 0.8783$ and ** correlation is significant at the 0.01 level (two-tailed), $p < 0.01 = 0.9587$.

Table 2-25 Correlation coefficients for seed quality and N-rate in cultivar 'Br-203'

Traits	**N-rate**	**FSP**	**TSW**	**PP**	**PSY**	**GP**
FSP	−0.2951					
TSW	−0.8789 *	−0.1906				
PP	−0.6613	−0.2844	0.7748			
PSY	0.1246	−0.6624	0.1416	0.6618		
GP	−0.6845	0.4739	0.4161	0.6840	0.2287	
PGSY	0.0716	−0.5615	0.1404	0.6945	0.9917 **	0.3415

Abbreviations: N-rate, rate of nitrogen application; FSP, filled seed percentage; TSW, thousand seed weight; PP, purity percentage; PSY, pure seed yield; GP, germination percentage; PGSY, pure germinated seed yield. Note: Values without asterisks are not significant, * correlation is significant at the 0.05 level (two-tailed), $p < 0.05 = 0.8783$ and ** correlation is significant at the 0.01 level (two-tailed), $p < 0.01 = 0.9587$.

Conclusions

This study was conducted to evaluate the effects of CCD and N-rate on seed yield and seed quality of two novel cultivars of *Urochloa* spp. (cv. 'OKI-1' and cv. 'Br-203') so that farmers can utilize information on the agricultural management of these grasses when they are promoted for planting in Northern Thailand or in other areas that have similar climatic conditions.

1. No significant mutual interactions were observed between the CCD and N-rate on seed yield, seed yield components, or seed quality in both cultivars.

2. The highest seed yields in cv. 'OKI-1' and cv. 'Br-203' were obtained when the CCD was 1 July and 15 July, respectively.

3. The CCD affected most seed quality attributes, except seed germination percentage (GP) in cv. 'OKI-1'.

4. The CCD in late-June to early-July and early-July to mid-July are recommended for cv. 'OKI-1' and cv. 'Br-203', respectively.

5. The seed yields in cv. 'OKI-1' and cv. 'Br-203' were non-significant in response to the different N-rates in the fertilization timing in our experiment.

CHAPTER 3

Effects of harvesting methods on seed yield and seed quality in new *Urochloa* cultivars

Abstract

Two field trials were conducted in 2018–2020 in Northern Thailand to determine an appropriate manual harvesting method for maximizing seed yield and seed quality of two new *Urochloa* cultivars, cv. 'Br-203' and cv. 'OKI-1'. Four manual seed harvesting methods were used in RCBD with four replicates. The four methods are: knocking seeds from seedheads into a nylon-net receptacle and collecting them once every day (T1), the same operation as T1 differs in collecting seeds once every three days (T2), allowing ripe seeds to fall into a nylon-net sheet that was stretched as a receptacle positioned beneath the seedheads, and collecting seeds once every five days (T3), and the tied seedheads were covered with a nylon-net bag and collected seeds once every five days (T4). The highest PSY was obtained from T3 in cv. 'Br-203', and it also showed the highest trend in cv. 'OKI-1'. T3 produced 22–46% and 11–27% more pure germinated seed yields than the other methods in cv. 'Br-203' and cv. 'OKI-1', respectively. As a result, T3 was recommended to achieve higher seed production, harvest seeds more conveniently, and reduce the cost and time spent on handling seed harvest.

Introduction

Seed harvesting is the process of taking the mature seed crop from the field with the purpose of obtaining a maximum seed yield while avoiding seed damage and quality deterioration through drying, cleaning, and delivery to storage (West & Pitman, 2001). It is a crucial process in seed production and is a critical operation for reaching the overall crop quality. A large amount of seed loss can occur both before and during the harvest. It is related to the adequacy of crop physiological maturity and seed moisture content. Therefore, it can be affected by the improperness of harvesting time and harvesting method (Benaseer et al., 2018).

Choosing an appropriate harvesting method and equipment for seed harvesting should be attentively considered. It can range from a hand operation to an absolutely mechanical one, depending upon the quantity of seed to be harvested, the type of product, planting and climatic conditions (West & Pitman, 2001). Additionally, it depends on the specific attributes of plant species, accessibility of machinery or employment, management skills of the farmer, and the value of the crop, which may also be altered by the market assortment. The tropical grass seeds can be harvested by a single destructive harvest of seed attached to the standing crop, multiple non-destructive harvests from the standing crop or ground recovery of ripe fallen seed from the standing crop (Loch & de Souza, 1999).

In the developing countries of the tropics, manual grass seed harvesting methods are performed predominately because of the low cost and accessibility to labor hire, including that it is suitable for a small planting area. Grass seed harvesting has been done manually in Thailand, as well as other tropical developing countries, for over four decades, ranging from single destructive harvests to multiple non-destructive harvests. For a single destructive harvest by hand harvesting, crops are either hand cut with sickles and rapidly threshed or cut and then sweated before threshing. However, some research papers in Thailand have reported that multiple non-destructive manual harvests can achieve higher seed yields than single destructive harvests (Hare et al., 2007b). Multiple-non-destructive manual seed harvesting methods, which comprised of tying groups of seedheads into living sheaves and knocking the seeds into broad shallow seed-net receptacles, covering and tying the seedheads with lightweight nylon net bags in order to allow mature seed to fall into these bags by shaking and collecting them at a few day intervals, including the use of a nylon net receptacle placed under the seedheads, were commonly used to maximize grass seed yields. Phaikaew & Pholsen (1993) found that daily knocking for collecting seeds of the *Urochloa ruziziensis* into seed-net receptacles obtained 50% more seed yield than cutting and sweating. The seed harvesting method of *Panicum maximum* TD.58 by covering the tied seedheads with a nylon net bag and collecting seeds every 3-5 days showed a higher seed yield and a thousand seed weight than the method of shaking the tied seedheads and collecting seeds every 3-5 days (792.5 kg/ha vs 571.9 kg/ha and 1.398 g vs 1.327 g, respectively) (Phaikaew et al., 1995). The highest pure seed yield (PSY) of *Panicum maximum* cv. 'Mombaza' was obtained from covering the seedheads with a nylon net bag and using a nylon net receptacle placed under the seedheads when compared with the shaking of seedheads into a large net receptacle every 3 days or daily (Phaikaew et al., 2010). In addition, the method of tying lightweight nylon net bags over seedheads produced substantially higher seed yields than knocking seedheads and ground sweeping for cv. 'Mulato' and cv. 'Mulato II' (Hare et al., 2007b). Even though the research of Kamphayae et al. (2013) found no significant difference in seed yield between seed harvesting methods, the highest seed germination percentage (91%) and TSW (1.352 g) of *Panicum maximum* cv. 'Umaku' was obtained by covering seedheads with a nylon net bag and collecting seeds one time, while the lowest was gained by the method of daily shaking seedheads.

Although manual seed harvesting methods are still widely used in these countries because they can produce high-quality commercial seeds through intensive collection, they face a number of challenges, including labor shortages and higher wages during the peak harvesting season, which coincides with rice harvesting, and adverse weather conditions are responsible for numerous seed losses caused by the necessity to harvest at an inappropriate harvesting time.

Making a choice of seed harvesting method to harvest two new *Urochloa* cultivars is very important for achieving good seed yields with high quality. To meet the reasonable price, an effective method of seed harvesting must be chosen in order to maximize seed production. To compete in price with other commercial *Urochloa* cultivars in South America, Hare et al. (2007b) claimed that seed yields must reach at least 500 kg/ha. In Thailand, achieving high seed yields and good quality *Urochloa* hybrids has been a challenge. Some *Urochloa* hybrids tested in Thailand failed to provide satisfactory seed yields. In seed production trials conducted by DLD in Northern Thailand, cv. 'Mulato II' produced significantly more seed (494 kg/ha) than cv. 'Mulato' (163 kg/ha) (Phunphiphat et al., 2007), but cv. 'Mulato' produced significantly more seed (331 kg/ha) than cv. 'Mulato II' (119 kg/ha) in Northeast Thailand (Nakmanee et al., 2007a). However, these yields were lower than seed yields of ruzigrass (3–4 times) in the same experiments.

Therefore, it was very interesting to find effective manual harvesting methods to maximize seed yield and seed quality and reduce these problems that have occurred. The main objective of this research was to determine a suitable multiple non-destructive hand harvesting method for seed production of two new *Urochloa* cultivars, cv. 'OKI-1' and cv. 'Br-203', in Northern Thailand, based on methods that had previously proven successful for other tropical grasses in Thailand.

Materials and methods

Location, soil characteristics, and meteorological data of the experimental sites

Initial soil samples were taken to a depth of 10 cm in May 2018, and post-harvest soil samples were taken in December 2019. The chemical properties of initial soil samples from field trial sites are shown in Table 3-1. The meteorological data, i.e., air temperature, rainfall, number of rainy days per annum, relative humidity, sunshine duration per day, and day length in 2018 and 2019 at the Lampang ANRDC site are shown in Figure 3-1 (A), (B), and (C).

Trial 1 – Effect of harvesting methods on seed yield and seed quality for cv. 'OKI-1'

Plant cultivation

Thirty-day-old seedlings of cv. 'OKI-1' were planted in the field on June 23, 2018, at a spacing of 1 × 1 m., using a plot size of 4 × 5 m. A basal fertilizer dressing (312.5 kg/ha, NPK 15%: 15%: 15%) was applied with an additional application of 28 kg N/ha as urea at planting time. One week after plantation, dead seedlings were replaced with fresh seedlings (grown at the same time as the dead seedlings) to obtain a uniform stand. Two months later, each tussock was tied together into manageable bunches with plastic ropes and loosely tied to a bamboo stake using a plastic-coated wire to avoid expanding the growth cover over the ground. The bamboo stake was fixed on the ground near each tussock. Weeds were controlled by manually removing them during the experiment. In the first year, the stand was established and no defoliation or cutting was performed. After the first seed harvest, all plots were cut to 10 cm above ground level to remove all of the forage on December 28, 2018. Within one day after cutting, the plant residues were manually removed. Then, topdressing urea was applied at 28 kg N/ha immediately. In the following year (2019), the grass was closed-cut on July 20 (the beginning of the rainy season). On September 11, each tussock was tied together into manageable bunches with plastic ropes and loosely tied to the bamboo stakes with plastic-coated wire, the same as in 2018.

Experimental design and treatments

A trial was performed using RCBD with four manual harvesting methods and four replicates. The treatments were as follows:

T1—knocking the seeds from seedheads into a broad, shallow nylon-net receptacle and collecting them once every day (Daily knocking);

T2—knocking the seeds from seedheads into a broad, shallow nylon-net receptacle and collecting them every three days (3 days-knocking);

T3—allowing ripe seeds to fall into a nylon net sheet stretched as a receptacle positioned beneath the seedheads and collecting them every five days (Under); and

T4—covering the tied seedheads with a light-weight nylon-net bag, allowing the ripe seeds to fall into the nylon-net bag, and collecting them every five days (Cover).

The seed harvesting methods used in the experiment are depicted in Figure 3-2 ((A), (B), and (C)).

Data collection and seed harvesting

All seedheads within each plot, except for the border row, were harvested over the period of seed maturity (late November–early December). The flowering and seed harvesting dates are shown in Table 3-2. TN/P was counted on three randomly selected plants in each plot at the time of harvest. IN/T was counted from 15 randomly selected tillers from the three selected plants. RN/I was counted from 15 randomly selected inflorescences of the three selected plants. SN/R was averaged from the upper, mid, and lower racemes of each inflorescence, which was randomly selected from the 15 inflorescences of the same three selected plants. (SN/R were counted only in 2019). Tiller fertility was expressed as the percentage of inflorescences over the total number of tillers. All the inflorescences in each plot were tied together into manageable bunches when the seed was almost ripe. Each seed harvesting method was carried out according to the experimental plan. For T1 and T2, ripe seeds were threshed off the inflorescence by lightly knocking them into the nylon-net receptacle when collecting seeds. For T3, the ripe seeds were shed into a nylon net sheet which was stretched as a receptacle placed under the seedheads when the first flower emerged. The nylon net sheets were tied together with bamboo stakes at 1 m above ground level. The plots were harvested individually.

Seed processing and calculation of secondary attributes

Seed processing and calculation of secondary attributes were done following the method described on page 68.

Statistical analysis

The data was analyzed and the estimated variance components in RCBD and the treatment means were tested by the LSD using R software version 4.1.0. (R Core Team, 2020), and significant differences among means were declared when $p < 0.05$.

Trial 2 – Effect of harvesting methods on seed yield and seed quality for cv. 'Br-203'

The experimental site of this trial was far from the experimental site of trial 1, approximately 30 m. The plant cultivation, experimental design and treatments, data collection and seed harvesting, seed processing and calculation of secondary attributes, and other management practices, including statistical analysis, for cv. 'Br-203' are the same as in trial 1.

Table 3-1 Chemical properties of initial soils of the experimental field in Trial 1 and Trial 2

Trial	pH	OM (%)	Total N (%)	Extractable (mg/kg)										EC (dS/m)
				P	K	Ca	Mg	Na	S	Mn	Fe	Cu	Zn	
1	5.22	0.71	0.04	2.72	51.71	74.44	11.45	3.38	2.45	19.12	54.48	0.19	0.02	0.41
2	5.33	0.49	0.02	0.64	81.13	278.42	43.53	2.44	2.75	10.78	46.91	0.15	0.06	0.30

Abbreviations: EC, electric conductivity; OM, organic matter.

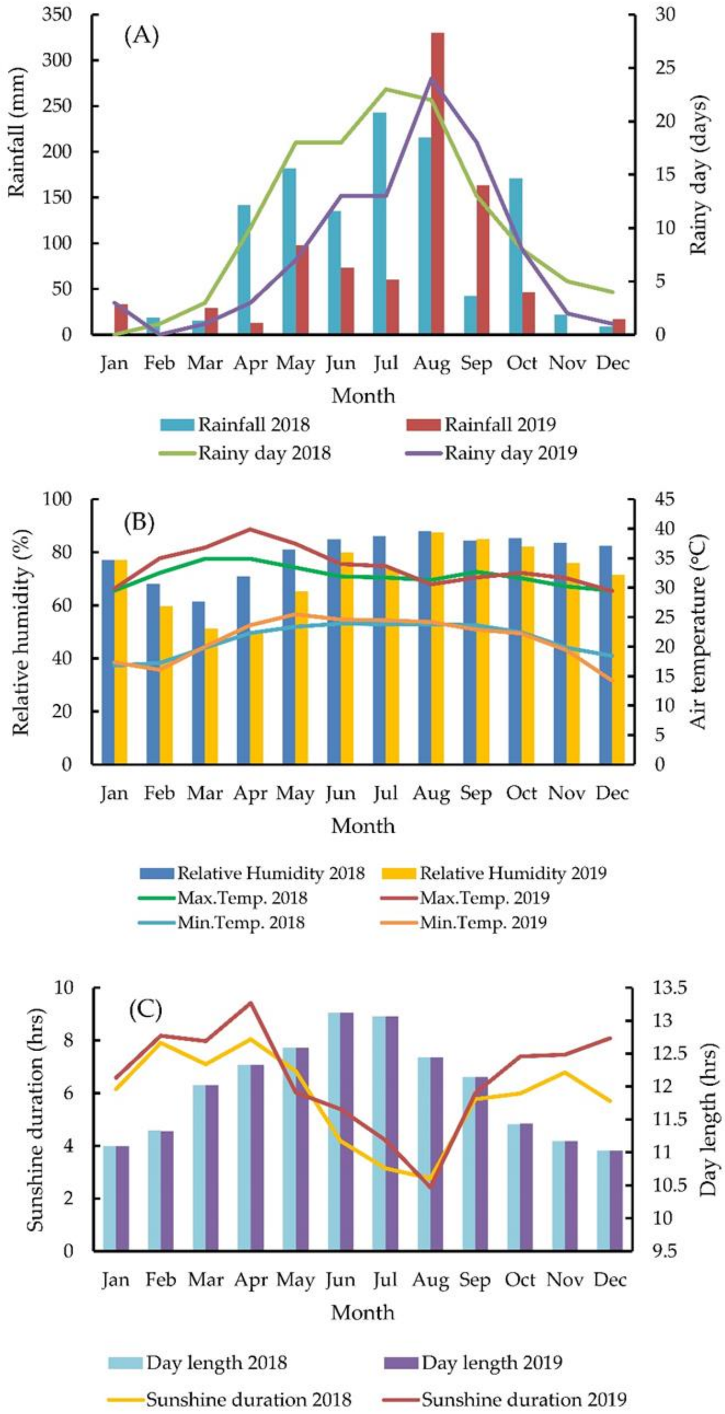

Figure 3-1 Meteorological data at the Lampang Animal Nutrition Research and Development Center in 2018 and 2019: (**A**) The average monthly rainfall and the number of rainy days in a month; (**B**) The monthly average relative humidity, the monthly average minimum, and the maximum temperature; (**C**) The monthly average day length and average daily sunshine duration.

Table 3-2 Dates of flowering and seed harvesting in both trials

Event	Year	
	2018	2019
Start of flowering	16 Oct	13 Oct
Period of harvesting	20 Nov–12 Dec	3 Dec–25 Dec
Post-harvest cut of herbage	16 Dec	30 Dec

(A) (B) (C)

Figure 3-2 Seed harvesting methods used in the experiment: (A) T1 and T2 (Knocking), (B) T3 (Under), and (C) T4 (Cover). The treatments were as follows:

T1—knocking the seeds from seedheads into a broad, shallow nylon-net receptacle and collecting them once every day (Daily knocking);

T2—knocking the seeds from seedheads into a broad, shallow nylon-net receptacle and collecting them every three days (3 days-knocking);

T3—allowing ripe seeds to fall into a nylon net sheet stretched as a receptacle positioned beneath the seedheads and collecting them every five days (Under); and

T4—covering the tied seedheads with a light-weight nylon-net bag, allowing the ripe seeds to fall into the nylon-net bag, and collecting them every five days (Cover).

Results

Trial 1 – Effect of harvesting methods on seed yield and seed quality for cv. 'OKI-1'

As shown in Tables 3-3 and 3-4, the different methods of seed harvesting did not have a significant effect on seed yield ($p < 0.05$) in each year of the experiment and the two-year average. The seed yields in 2018 were almost twice as many as in 2019. The highest TSY, PSY, and PGSY tended to be obtained from T3 in 2018 and T2 in 2019. However, the tendency for the highest seed yield of the two-year average was obtained from T3. Several seed yield components were variable over the two years of the experiment. In 2018, and on a two-year average, a large number of IN and IN/T were achieved from T1. The lower TN, IN, and IN/T were obtained in the second year of the experiment when compared with the establishment year, in contrast to FTN, FTP, and RN/I. Most seed quality attributes, except PP, were not influenced by the harvesting method. The lowest PP was found in T1. The PP and TSW were lower in 2019 when compared to in 2018. In contrast, a higher GP was observed in 2019. The FSP and TSW of T1 tended to be the lowest in both years of the experiment. T1 resulted in the significantly lowest PP in both 2019 and the two-year average, and it also tended to be the lowest in 2018.

Trial 2 – Effect of harvesting methods on seed yield and seed quality for cv. 'Br-203'

As shown in Tables 3-5 and 3-6, a significant effect of the seed harvesting methods on seed quality was not detected ($p < 0.05$). Although a significant difference between treatments for TSY in the two-year average and PSY in both 2019 and the two-year average was found, there was no significant difference in PGSY in each year of the experiment and in the two-year average. The seed harvesting method influences some seed yield components that vary from year to year. A significant effect of the seed harvesting methods on TN and FTN was found ($p < 0.05$) in 2018, and a significant difference in IN and IN/T was detected ($p < 0.05$) in 2019. In the first year of the experiment, T1 had significantly more TN than T2 and T3, and markedly more FTN than T3. However, a significant difference between treatments in all seed yield components was not found in the two-year average. In both years of the experiment, the harvesting methods had no effect on all seed quality attributes (i.e., FSP, TSW, PP, GP).

Table 3-3 Effect of harvesting method on seed yield and its components in cultivar ‘OKI-1’

Year	Harvesting method	TSY (kg/ha)	TN (no./m^2)	FTN (no./m^2)	IN (no./ m^2)	FTP (%)	IN/T (no.)	RN/I (no.)	SN/R (no.)
2018	T1 ‘Daily knocking’	579.96	84.17	55.50	238.18[a]	65.72	4.32[a]	2.86	-
	T2 ‘3 days-knocking’	660.42	88.58	58.33	182.26[ab]	65.40	3.12[b]	3.26	-
	T3 ‘Under’	764.83	72.83	45.83	118.38[b]	62.32	2.57[b]	3.34	-
	T4 ‘Cover’	669.60	75.75	50.17	144.30[b]	66.14	2.90[b]	3.35	-
2019	T1 ‘Daily knocking’	253.26	80.25	78.00	115.83	97.31	1.48	4.17	36.50
	T2 ‘3 days-knocking’	362.18	78.50	75.42	119.98	96.08	1.60	4.19	35.64
	T3 ‘Under’	307.68	69.83	68.50	105.73	97.83	1.48	4.34	40.17
	T4 ‘Cover’	311.23	74.42	73.08	101.23	98.15	1.35	4.24	34.25
2-year average	T1 ‘Daily knocking’	416.61	82.21	66.75	177.00[a]	81.51	2.90[a]	3.52	-
	T2 ‘3 days-knocking’	511.30	83.54	66.88	151.12[ab]	80.74	2.36[b]	3.72	-
	T3 ‘Under’	536.25	71.33	57.17	112.06[b]	80.07	2.02[b]	3.84	-
	T4 ‘Cover’	490.42	75.08	61.62	122.77[b]	82.14	2.12[b]	3.80	-

Abbreviations: TSY, total seed yield; TN, tiller number per square meter; FTN, fertile tiller number per square meter; IN, inflorescence number per square meter; FTP, fertile tiller percentage; IN/T, inflorescence number per tiller; RN/I, raceme number per inflorescence; SN/R, spikelet number per raceme; ns, non-significant. Note: Values with different superscript letters indicate statistical differences within each column for each year and the 2-year average ($p < 0.05$).

Table 3-4 Effect of harvesting method on seed quality in cultivar 'OKI-1'

Year	Harvesting method	FSP (%)	TSW (g)	PP (%)	PSY (kg/ha)	GP (%)	PGSY (kg/ha)
2018	T1 'Daily knocking'	32.03	9.12	99.72	578.41	81.75	473.06
	T2 '3 days-knocking'	40.12	9.60	99.82	659.23	77.25	528.97
	T3 'Under'	40.65	9.53	99.78	763.14	88.00	683.61
	T4 'Cover'	32.19	9.42	99.88	668.74	77.75	519.67
2019	T1 'Daily knocking'	32.67	8.21	97.78^{b}	246.64	96.25	236.65
	T2 '3 days-knocking'	33.28	8.76	98.62^{ab}	357.49	95.00	339.95
	T3 'Under'	38.14	8.91	99.50^{a}	306.20	95.75	293.39
	T4 'Cover'	35.67	8.85	99.78^{a}	310.53	92.50	287.25
2-year average	T1 'Daily knocking'	32.35	8.66	98.75^{b}	412.53	89.00	354.85
	T2 '3 days-knocking'	38.76	9.18	99.22^{ab}	508.36	86.12	434.46
	T3 'Under'	39.40	9.22	99.64^{a}	534.67	91.88	488.50
	T4 'Cover'	33.35	9.13	99.82^{a}	489.64	85.12	403.46

Abbreviations: FSP, filled seed percentage; TSW, thousand seed weight; PP, purity percentage; PSY, pure seed yield; GP, germination percentage; PGSY, pure germinated seed yield; ns, non-significant. Note: Values with different superscript letters indicate statistical differences within each column for 2019 and the 2-year average ($p < 0.05$).

Table 3-5 Effect of harvesting method on seed yield and its components in cultivar 'Br-203'

Year	Harvesting method	TSY (kg/ha)	TN (no./m^2)	FTN (no./m^2)	IN (no./ m^2)	FTP (%)	IN/T (no.)	RN/I (no.)	SN/R (no.)
2018	T1 'Daily knocking'	277.10	68.42^{a}	52.00^{a}	218.12	76.27	4.17	3.27	-
	T2 '3 days-knocking'	257.28	54.00^{b}	40.25^{ab}	178.64	73.48	4.38	3.27	-
	T3 'Under'	417.89	53.08^{b}	38.92^{b}	163.34	72.77	4.25	3.17	-
	T4 'Cover'	379.15	62.75^{ab}	45.83^{ab}	172.69	73.06	3.75	2.79	-
2019	T1 'Daily knocking'	136.80	79.00	71.08	85.50^{b}	88.53	1.22^{c}	4.88	34.40
	T2 '3 days-knocking'	160.67	94.92	82.42	143.88^{ab}	86.80	1.70^{b}	4.08	36.62
	T3 'Under'	268.37	83.67	77.25	155.45^{ab}	92.40	2.12^{a}	4.33	33.66
	T4 'Cover'	197.61	101.83	90.92	164.27^{a}	89.67	1.80^{ab}	4.05	32.54
2-year average	T1 'Daily knocking'	206.94^{b}	73.71	61.54	151.81	83.08	2.69	4.07	-
	T2 '3 days-knocking'	208.97^{b}	74.46	61.33	161.26	81.96	3.04	3.63	-
	T3 'Under'	343.13^{a}	68.38	58.08	159.40	84.82	3.18	3.80	-
	T4 'Cover'	288.38^{ab}	82.29	68.38	168.48	83.39	2.78	3.42	-

Abbreviations: TSY, total seed yield; TN, tiller number per square meter; FTN, fertile tiller number per square meter; IN, inflorescence number per square meter; FTP, fertile tiller percentage; IN/T, inflorescence number per tiller; RN/I, raceme number per inflorescence; SN/R, spikelet number per raceme; ns, non-significant. Note: Values with different superscript letters indicate statistical differences within each column for each year and the 2-year average ($p < 0.05$).

Table 3-6 Effect of harvesting method on seed quality in cultivar 'Br-203'

Year	Harvesting method	FSP (%)	TSW (g)	PP (%)	PSY (kg/ha)	GP (%)	PGSY (kg/ha)
2018	T1 'Daily knocking'	28.93	6.94	98.52	273.46	34.50	90.17
	T2 '3 days-knocking'	24.28	7.28	98.12	252.93	35.00	87.11
	T3 'Under'	17.53	6.89	97.52	407.70	41.12	180.71
	T4 'Cover'	14.62	6.96	98.10	373.79	39.19	140.27
2019	T1 'Daily knocking'	26.72	6.51	97.15	133.05^{b}	94.25	125.44
	T2 '3 days-knocking'	12.26	6.49	97.42	156.52^{ab}	95.25	148.98
	T3 'Under'	15.33	7.45	96.70	258.52^{a}	84.25	219.55
	T4 'Cover'	10.57	6.69	96.32	190.61^{ab}	89.75	170.82
2-year average	T1 'Daily knocking'	27.83	6.72	97.84	203.26^{b}	64.38	107.80
	T2 '3 days-knocking'	18.27	6.88	97.78	204.72^{b}	65.12	118.04
	T3 'Under'	16.43	7.17	97.11	333.11^{a}	62.69	200.13
	T4 'Cover'	12.59	6.83	97.21	282.20^{ab}	64.47	155.54

Abbreviations: FSP, filled seed percentage; TSW, thousand seed weight; PP, purity percentage; PSY, pure seed yield; GP, germination percentage; PGSY, pure germinated seed yield; ns, non-significant. Note: Values with different superscript letters indicate statistical differences within each column for 2019 and the 2-year average (p < 0.05).

Discussion

Although the soils at both study sites were acidic, with low organic matter content (less than 1%) and deficits in essential nutrients, cv. 'OKI-1' and cv. 'Br-203' demonstrated the potential for edaphic adaptation in growth and productivity (Table 3-1). Most commercial *Urochloa* species are adaptable to a wide variety of soil types, from low-fertility acid soils to high-fertility neutral soils, in accordance with the report by Rao et al. (1996).

The report by Nadew (2018) concluded that rainfall and temperature are some of the key climatic conditions that strongly affect the state of the physiological processes in seeds and eventually affect the yield and quality of seeds. The important role of temperature is the same as rainfall. According to Bouathong et al. (2011), the optimum temperature and RH for *Urochloa* species were 25°C–35°C and 60%–70%, respectively. The average air temperature and RH throughout the year (2019) were 27.4°C and 71.6%, respectively. They were in a suitable range, though the RH was a bit higher.

Apart from the factors mentioned above, the sufficiency of solar radiation and favorable photoperiods at each stage of the growth cycle, including dry weather during maturation and harvest, are also factors affecting the production of forage seed crops. At the experimental sites, the average day length was approximately 12 hours/day (11 hours/day in December to 13 hours/day in June) and the sunshine duration averaged 6.6 hours/day, with the shortest in August (approximately 3 hours/day) and the longest in April (approximately 9 hours/day). This complies with the report by Wongsuwan (1994) that critical day length triggers reproductive development of *U. ruziziensis* in Thailand, approximately 12 ½ hours, and found that the greatest seed yield was obtained from the shorter daylength (11 hours) than the other longer daylengths (14, 13 and 12 hours). Northern Thailand's climatic conditions, particularly little rainfall, low humidity, and low temperatures from flowering to seed ripening, are ideal for cultivars 'OKI-1' and 'Br-203' seed harvesting.

In our trials, only cv. ‘OKI-1’ produced PSY at more than 500 kg/ha by T3 and T2 methods. The ‘Under’ method (T3) tended to produce higher seed yields than the ‘Knocking seedheads’ (T1 and T2) and the ‘Cover’ method (T4) for both cv. ‘OKI-1’ and cv. ‘Br-203’.

Although the method of seed harvesting had no significant effect on the seed yield of cv. ‘OKI-1’, allowing the ripe seeds to shed into a nylon net sheet which was stretched as a receptacle positioned beneath the seedheads and collecting seeds every five days (T3) brought a tendency to increase more seed yields than the other methods. The ‘Under’ method increases 12–16% more seed yields than the others. In line with a previous study on the seed harvesting method of *Paspalum atratum* in Thailand, the highest PSY was obtained from the use of a nylon-net receptacle placed under the seedheads (the ‘Under’ method) and the use of a nylon-net bag covering the seedheads (the ‘Cover’ method). Though there was no significant difference between the two methods, the ‘Under' method produced almost 12.5% more PSY than the ‘Cover' method (Phaikaew et al., 2001b). This contrasted with the study on *Paspalum atratum* which was conducted by Kowithayakorn & Phaikaew (1993) and Hare et al. (1999), where the highest seed yields were obtained from knocking ripe seed daily into bags. Due to the mature seeds’ spontaneously shedding into a nylon-net sheet without disturbing management during seed harvesting, the ‘Under’ method (T3) gave the highest seed yield compared to the other methods. Furthermore, the ‘Under' method avoids harvesting immature seeds and allows them to continue ripening on the plant. In the second year of both trials, seed yields were found to be lower, at about half of the first year, though N fertilizer was applied. As proven by Gelderman et al. (1987), seed yields can decline after the year of establishment, with or without N application.

In the case of cv. ‘Br-203’, the ‘Knocking’ method (T1 and T2) brought significantly lower TSY and PSY compared to the ‘Under’ method (T3), though the significant difference ($p < 0.05$) between the ‘Cover’ method (T4) was not detected. The ‘Under’ method (T3) increases 22–46% more seed yields than the other methods. One reason for the lower seed yield in the “Knocking” method of our study may be that the immature seeds would be shed from the seedheads apart from the fully mature seeds during harvest. The significant differences ($p < 0.05$) in TN and FTN were found in the establishment year (2018) because the closing cut had not occurred, resulting in the variation in the tiller number. In spite of the fact that the overall TN, FTN, RN/I, and FTP in 2018 were lower than in 2019, TSY and PSY were obtained more. It could be caused by the significant decrease in IN and IN/T, as well as the decrease in FSP, TSW, and PP. The TSW was slightly lower in 2019 than in 2018, except in the ‘Under’ method. The tendency of the highest PGSY was obtained from the ‘Under’ method, as the same as in the case of cv. ‘OKI-1’. With this method, only ripe and completely mature seeds would be harvested without the damage caused by harvesting. However, the ‘Under' and ‘Cover' methods appear to provide higher seed yields than the ‘Knocking’ method. Similar results were obtained with *Paspalum atratum* (Phaikaew et al., 2001b).

The seed yields in 2019 decreased almost twice from 2018. Apart from the reduction of nutrients in soil by plant assimilation throughout the two-year experiment, the poor seed yields could be related to high-temperature stress and inadequate rainfall during the seed filling period (from flowering until the end of seed ripening). Sehgal et al. (2018) validated this finding by stating that drought and heat stress have a significant impact on seed yields by reducing seed size and quantity, consequently affecting seed weight and quality. In addition, seed filling is regulated by several metabolic processes occurring in the leaves, especially production and translocation of photoassimilates, which import precursors for biosynthesis of seed reserves, minerals, and other functional elements. These processes are extremely sensitive to drought and heat due to the involvement of an array of various enzymes and transporters located in the leaves and seeds. Browning and dieback of leaves and stems, as well as the premature death of spikelets, were discovered in some clumps of grass plants. Furthermore, the most considerable increase in GP occurred in 2019 compared to 2018, particularly in cv. 'Br-203'. The lower GP in 2018 might have been caused by the higher RH since flowering until harvesting time, continuing rain until harvested, and shorter sunshine duration during the drying process, which resulted in mold occurring in some seeds during germination testing.

The results of this study recommend that allowing ripe seeds to fall into a nylon net sheet stretched as a receptacle positioned beneath the seedheads and collecting them every five days (T3) is suitable for manual seed harvesting in all two new *Urochloa* cultivars. Aside from obtaining a higher seed yield, the T3 method is also easier to handle seed collection, less time-consuming, and minimizes labor costs. Despite the fact that this method is at risk of being destroyed by pests (i.e., insects, birds, and rodents), frequent seed harvest is likely to mitigate the problem. Additionally, this method could avoid seedhead damage caused by covering them with a nylon-net bag or directly knocking the seedheads.

Conclusion

Allowing ripe seeds to fall into a nylon net sheet stretched as a receptacle positioned beneath the seedheads, 1 m above the ground, and collecting them every five days or more frequently is the hand seed harvesting method suitable for all two new *Urochloa* cultivars. The method was practical and recommended in order to achieve more seed yields. Furthermore, it facilitates seed collection, lowering the cost and time spent on seed harvesting.

CHAPTER 4

Study on the water imbibition of four *Urochloa* cultivars' seeds

Abstract

This study investigated the seed imbibition of four *Urochloa* cultivars, emphasizing the absorption of water by their intact seeds. All of these seeds had poor imbibition capacity. The uptake of water takes place within the first two hours, after which this uptake becomes very slow. The increase patterns of water uptake percentage (WUP) in the cultivars used were fitted to logarithmic curves. As for the increase rate, a difference was accepted between cultivars. Throughout the imbibition time, the significant highest average WUP was observed in *U. decumbens* cv. 'Basilisk' (26.1%), followed by *U. ruziziensis* cv. 'OKI-1' (22.9%), and *U. ruziziensis* cv. 'Br-203' (22.5%), while *U. ruziziensis* cv. 'Kennedy' showed the lowest (18.9%). The results revealed that the seed coat of the four cultivars is not an obstacle to water penetration into the embryo. Therefore, the seed-covering part of these *Urochloa* spp. does not impede water absorption, which leads to being the cause of seed dormancy as well as obstructing germination. The imbibition percentage, i.e., water uptake speed of two novel *Urochloa* cultivars, cv. 'OKI-1' and cv. 'Br-203', are similar to cv. 'Basilisk' and cv. 'Kennedy', which are widely used in tropical regions.

Introduction

In the tropical zone, water acts as a major limiting factor for the germination of seeds because of erratic rainfall. Water absorption is the first critical step in the chain of events that leads to germination. Germination begins only when the seed reaches a certain level of hydrature for metabolic activity, and any seed that remains unimbibed does not germinate (Bansal et al., 1980). Apart from oxygen and a favorable temperature, water is one of the primary requirements for the germination of seeds (Burch & Delouche, 1959). Water acts as a colloidal solvent, allowing for an increase in enzyme synthesis/reactions, while the increased pressure caused by colloidal swelling aids in seed coat rupture. (Mayer & Poljakoff-Mayber, 1989).

Imbibition, which is the adsorption of water by nonliving or senescent materials and subsequent swelling caused by adhesion of the water to the internal surfaces of materials, is the initial step in germination (Bradford, 1990; 1995). The imbibition depends upon the composition of the seed, the permeability of the seed coat to water, and the availability of moisture. Even under favorable conditions, seeds surrounded by an impermeable seed coat will not swell (Bansal et al., 1980). Plant seeds are low in moisture (5–15%) and almost metabolically inactive when they are in a resting state (Louf et al., 2018). It is essential for enzyme activation, the breakdown of starch into sugars, and the transport of nutrients to the developing embryo. The imbibition of water is considered to be an induced flow (the amount of which is known as the hydrodynamic mass) through pores in the seed coat rather than a passive diffusion of water.

The aim of this research was to evaluate by comparing the seed imbibition of two commercial *Urochloa* cultivars (cv. 'Kennedy' and cv. 'Basilsk') and two novel cultivars, i.e., cv. 'OKI-1' and cv. 'Br-203'.

Materials and Methods

Seed Materials

The matured seeds of four *Urochloa* spp. used for this study were as follows:

1) *U. ruziziensis* (R. Germ. and C.M. Evrard) Crins. cv. Kennedy (abbr. cv. 'Kennedy')

2) *U. decumbens* (Stapf) R.D. Webster cv. Basilisk (abbr. cv. 'Basilisk')

3) *U. ruziziensis* (R. Germ. and C.M. Evrard) Crins. cv. OKI-1, which was open pollinated by chromosome doubling (abbr. cv. 'OKI-1')

4) *U. ruziziensis* (R. Germ. & C.M. Evrard) Crins × *U. brizantha* (Hochst. Ex A. Rich.) Stapf, cv. Br-203 (abbr. cv. 'Br-203')

All the seeds were collected from the Lampang ANRDC, Lampang, Thailand. After harvest in December 2018, the seed was air-dried for several days in the shade before being sun-dried for a few days and then cleaned using a hand screen. Seeds with endosperm (filled seeds) were separated from the inert fraction and empty or light seeds using a South Dakota seed blower. Then these seed samples were stored dry in a paper bag and kept in the refrigerator (5°C). Seed imbibition testing commenced in March 2019, and was conducted at the Faculty of Agriculture, the University of the Ryukyus, Okinawa, Japan.

Determination of Water Uptake in Intact Seeds

Imbibition testing was carried out using four replicates of 100 seeds. The cleaned, dried seeds were weighed and thereafter placed in test tubes and immersed in distilled water (10 ml) and incubated individually for 2, 4, 6, 8, and 24 hours at 25°C ± 2°C. After the desired time, the seeds were wiped dry with blotting paper and weighed. The water uptake was expressed as an increase over the initial seed weight. The water uptake percentage (WUP) was defined by the following equation, where W_n is the seed weight at n hours after imbibition and W_i is the seed weight before imbibition started:

$$WUP\ (\%) = 100\ [(W_n - W_i)/\ W_i]$$

Experimental Design

The treatments were arranged according to a factorial scheme (5×4) in which the first factor was the duration of imbibition (2, 4, 6, 8, and 24 hours) and the second factor was the four cultivars. The experiment was conducted as a completely randomized design (CRD) with four replicates.

Statistical Analysis

The ANOVA was performed using a factorial design in CRD consisting of four *Urochloa* cultivars and five imbibition periods with four replicates. Means were compared by the LSD test at a probability level of 0.05 using the R software version 4.1.0 (R Core Team, 2021).

Results

The data on the amount of water uptake (g/100 seeds) in seeds is presented in Table 4-1. The seeds of different grass cultivars absorbed varying amounts of water at various imbibition time periods. The seeds in each cultivar have the highest amount of water absorption at 24 hours after being immersed. The cultivar 'OKI-1' seeds showed the highest amount of water absorption, followed by cv. 'Br-203', cv. 'Basilisk', and cv. 'Kennedy' seeds, respectively. Intact seeds of all cultivars can absorb water within the first 2 hours of immersion. Afterward, the water uptake into the seeds was very low.

Table 4-2 shows the interaction between *Urochloa* spp. cultivars and imbibition times on water uptake percentage. Within the first two hours, the average value of WUP in all grass cultivars was the lowest. The cv. 'Basilisk' showed maximum WUP within the first six hours, after which the water uptake became slow. The highest WUP of most grass cultivars, except for cv. 'Basilisk', was shown at 24 hours of imbibition. Throughout 24 hours of imbibition, the average percentage of water uptake was highest in the seeds of cv. 'Basilisk' (26.12%), followed by cv. 'OKI-1' (22.95%) and cv. 'Br-203' (22.53%), while cv. 'Kennedy' (18.95%) was the lowest.

As for the increase rate, a difference was accepted between cultivars. The changes in WUP of the *Urochloa* spp. with the progress of imbibition time are shown in Figure 4-1. The increase patterns of WUP in the cultivars used with the passage of imbibition time were fitted to logarithmic curves. As for the increase rate, a difference was accepted between cultivars.

Table 4-1 Interaction between the cultivar of *Urochloa* spp. and imbibition duration on the amount of water uptake (g/100 seeds)

Imbibition time (hrs.)	Amount of water uptake (g/100 seeds)				Mean
	Kennedy	Basilisk	OKI-1	Br-203	
2	0.108	0.132	0.163	0.172	0.143^{d}
4	0.122	0.134	0.209	0.190	0.164^{bc}
6	0.104	0.166	0.223	0.145	0.159^{c}
8	0.120	0.174	0.235	0.151	0.170^{b}
24	0.143	0.173	0.291	0.198	0.201^{a}
Mean	0.119^{D}	0.156^{C}	0.224^{A}	0.171^{B}	

Note: Mean values followed by different superscript letters in the same row (capital letter) and column (small letter) indicate statistical differences ($p < 0.05$).

Table 4-2 Interaction between the cultivar of *Urochloa* spp. and imbibition duration on water uptake percentage (%)

Imbibition time (hrs.)	Water uptake percentage (%)				Mean
	Kennedy	Basilisk	OKI-1	Br-203	
2	16.424	23.170	17.337	19.489	19.105^{d}
4	19.963	24.067	20.420	21.341	21.448^{c}
6	16.867	28.432	22.200	21.315	22.203^{bc}
8	19.178	27.361	24.300	22.363	23.301^{b}
24	22.314	27.548	30.494	28.158	27.129^{a}
Mean	18.949^{C}	26.116^{A}	22.950^{B}	22.533^{B}	

Note: Mean values followed by different superscript letters in the same row (capital letter) and column (small letter) indicate statistical differences ($p < 0.05$).

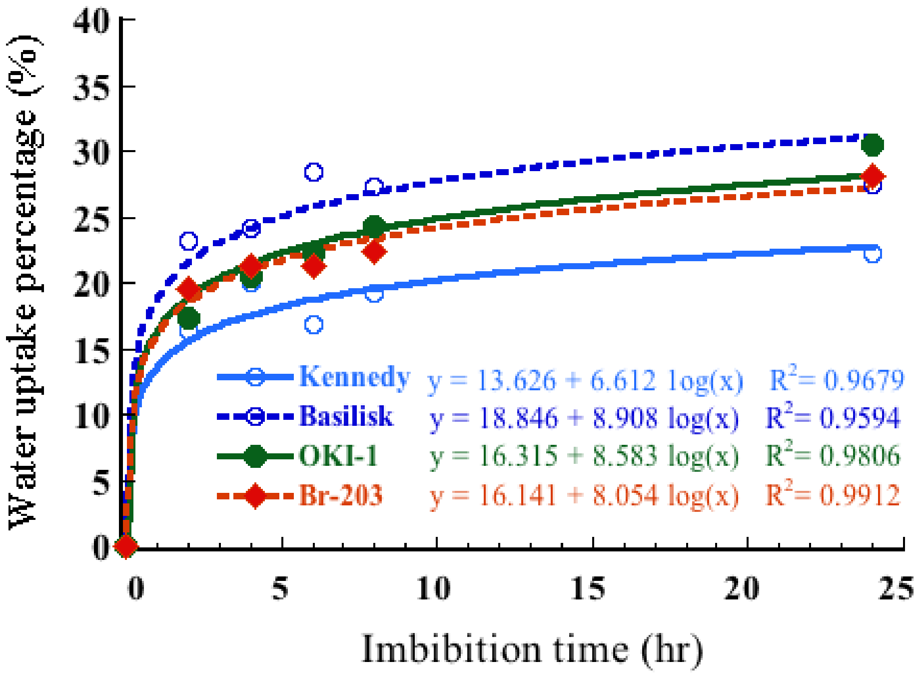

Figure 4-1 The water uptake percentage of the cultivars of *Urochloa* spp. changes with the progress of imbibition time.

Discussion

Successful germination and seedling development are crucial steps in the growth of a new plant. Seeds of most tropical grasses show seed dormancy, including *Urochloa* genotypes (Simpson, 1990; Adkins et al., 2002). Seed dormancy can be divided into two main components: coat and embryo dormancy. The coat component could be due to the effect of external (floral) covering structures and/or caryopsis coats. Dormant seeds with a coating physically develop an opening where water can enter the seeds (Baskin & Baskin, 2004).

This difference in the rate of water absorption by the seeds of four *Urochloa* cultivars probably resulted from the specific characteristics of the cultivars, though they are in the same genus. Seed coat characteristics probably also contributed to the variation in the rate of absorption by the different kinds of seed. The uptake of water by seeds is an essential early step in germination. Rates of water uptake are critical to successful germination; if uptake is too slow, germination is reduced because seed may deteriorate. If uptake is too rapid, seeds may exhibit imbibitional damage (Mehrafarin et al., 2011).

The results revealed that in four *Urochloa* cultivars used in this experiment, the seed-covering part does not impede water penetration to the embryo. Therefore, the seed-covering part of these *Urochloa* spp. does not impede water absorption, which leads to being the cause of seed dormancy as well as obstructing germination.

Conclusion

The seed-covering part of four *Urochloa* spp. does not impede water absorption. The imbibition percentage, i.e., water uptake speed of two novel *Urochloa* cultivars, cv. 'OKI-1' and cv. 'Br-203', are similar to commercial *Urochloa* cultivars, cv. 'Basilisk' and cv. 'Kennedy', which are widely used in tropical regions.

Effects of sulfuric acid (H_2SO_4) scarification, and priming with potassium nitrate (KNO_3), and gibberellic acid (GA_3) on seed dormancy breaking and promoting germination in *Urochloa ruziziensis* (cv. 'OKI-1')

Abstract

Poor seed germination caused by dormancy has been a major issue in the use of many desirable tropical range grasses. The objective of this study was to evaluate the effectiveness of different methods to break the dormancy of new *Urochloa ruziziensis* (cv. 'OKI-1') seeds stored at 5°C and 25°C. The four-month-old seeds were submitted to the following methods to break dormancy and promote germination: soaking the seeds in concentrated (36N) H_2SO_4 for 0, 2, 4, 8, 16, and 24 minutes; and then washing and drying the seeds before moistening the germination substrate with 0.2% KNO_3, 100 ppm GA_3, and DW. The experimental design was completely randomized in a 6 × 3 factorial scheme. After the complete germination (21 days), the seed germination percentage was quantified. Soaking seeds in H_2SO_4 for 8 to 16 minutes before sowing in substrate containing 100 ppm GA_3 was an effective treatment for breaking dormancy and increasing germination in seeds of cv. 'OKI-1' stored at 5°C and 25°C.

Introduction

Urochloa species typically exhibit a double seed dormancy mechanism, which is characterized by dormancy primarily on fresh-harvested seeds, resulting in germination percentages lower than those of viability detected by TZ and causing issues in storage, trading, and seed inspection activities (Filho & Usberti, 2008). Most grass seeds possess post-harvest dormancy and require some period of storage for the release of dormancy (Whiteman & Mendra, 1982). However, caryopses lose their viability faster than spikelets under storage. Seeds stored at low temperatures germinate at a slower rate than seeds stored at room temperature (Geetha, 2001).

A rapid germination rate is essential for successfully establishing forage grass pasture fields. However, seed dormancy may aid in the establishment and persistence of the stand, as well as the avoidance of germination immediately following harvest (Herrera, 1994). The main causes of that dormancy are the impermeability of the tegument to O_2 as well as the embryonic dormancy. Conditions stimulating dormant seeds to germinate were found to include soaking in water, cutting of the seed coat, acid scarification, mechanical scarification, subjection to alternating temperatures, removal of hulls, soaking in KNO_3 and in ammonium thiocyanate, and after-ripening at warm temperatures (Akamine, 1944). Several methodologies for releasing dormancy from those seeds have been tested, primarily using H_2SO_4 scarification (Filho & Usberti, 2008). The mechanical method and scarification with H_2SO_4 are the most efficient in overcoming dormancy (Souza et al., 2021). Seeds scarified with H_2SO_4 recorded high germination in *U. brizantha* (Montorio et al., 1997; Castro et al., 1994). McLean & Grof (1968) found that the acid scarification of *U. ruziziensis* seeds was more efficient in releasing dormancy than mechanical scarification, implying that low pH could also affect the physiological dormancy of seeds in the genus *Urochloa*.

Priming involves the partial moistening of seeds, which helps to unlock the metabolic processes that occur during the early stages of germination, preventing radicle protrusion and maintaining seed tolerance to desiccation (Paparella et al., 2015; Ibrahim, 2016). As a result, the seeds prepare for germination by restructuring membranes and reorganizing metabolic systems, which favors faster uniform germination when conditions become more favorable or in adverse environments (Jisha et al., 2013; Ibrahim, 2016; Batista et al., 2018). KNO_3 and GA_3 are commonly used to break seed dormancy and promote seed germination (Gashi et al., 2012). According to the scientific literature on seed priming, KNO_3 has shown promising results in improving the physiological quality such as seedling length, radical length, and seedling dry weight of several species (Anosheh et al., 2011; Entesari et al., 2012), including *U. brizantha* (Bonome et al., 2006; Binotti et al., 2014; Cardoso et al., 2015). Nitrate priming has been shown in studies to overcome dormancy and increase germination because they act as oxidants, converting NAD(P)H to NAD(P)+ in the pentose-phosphate metabolic pathway (Hendricks & Taylorson, 1974; Cardoso et al., 2015). Soaking seeds in KNO_3 solution effectively releases the seed dormancy of *Urochloa* spp. (Faria et al., 1996), *U. humidicola* (Libório et al., 2017; Pereira et al., 2021), and *U. decumbens* (Herrera, 1994). GA_3 is also effective for the germination improvement of seeds of several grass species (Parihar et al., 1999; Shahi & Sen, 1991; Usberti & Valio, 1997; Ma et al., 2018), including *U. brizantha* (Vieira et al., 1998; 1999).

Seeds scarified with H_2SO_4, dried and again soaked in KNO_3 solution with alternating temperature released dormancy in several grass species were recommended (Smith, 1971; Basra et al., 1990; Previero et al., 1996). Seeds also showed improvement in germination with acid scarified and soaked in GA_3 solution (Hongru et al., 1995; Singh et al., 1995). The seed may require different types of treatment to break seed dormancy and to make the seed readily germinative in the forthcoming seasons. Seed dormancy breaking treatment can be given to the seed based on the type and place of seed dormancy. (Shanmugavalli et al., 2007).

Based on the results of this study, due to cv. 'OKI-1' exhibited more seed production performance than cv. 'Br-203' and is viable for the commercialized seed market, cv. 'OKI-1' was selected for further study on overcoming seed dormancy. Creating optimal conditions for the germination of cv. 'OKI-1' seed is essential for its cultivation. Therefore, this study aimed to evaluate the effectiveness of acid scarification treatments and the application of germination promoters on the dormancy breaking of stored seeds at two levels of storage temperatures for the candidate *Urochloa ruziziensis* (cv. 'OKI-1').

Materials and Methods

The experiment was conducted in the Faculty of Agriculture at the University of the Ryukyus, Okinawa, Japan in April 2020. The cv. 'OKI-1' seeds were hand-harvested from a cultivated field at the Lampang ANRDC, Northern Thailand, in December 2019. They were dried to about 8% seed moisture content, cleaned, immature, and damaged seeds were removed, then kept in a plastic zip bag, and brought to Japan in February 2020. The seeds were divided into 20 g lots and placed in 36 plastic zip bags. Put the 18 bags in a paper bag and stored them in a room that was maintained at a temperature of 25°C and average daily RH was in a range of 65% to 78%, and the other 18 bags were placed in another paper bag and stored in a fridge (5°C, 20% RH).

The experimental design was completely randomized in a 6 × 3 factorial arrangement, consisting of 6 duration times to be soaked with concentrated (36 N) H_2SO_4: 0, 2, 4, 8, 16, and 24 minutes; and 3 substrate solutions for priming seed: 0.2% KNO_3, 100 ppm GA_3), and DW, with 4 replicates. The seeds were soaked with concentrated (36N) H_2SO_4 for a specified period, then washed in running cold water and dried under a fan overnight to bring the SMC to about 10%. For the germination test, four lots of 100 seeds for each treatment were drawn and placed inside transparent plastic petri dishes with a 10 cm diameter on top of filter paper (Whatman no.1) moistened with specified germination promoter treatments: 0.2% KNO_3, 100 ppm GA_3, and DW. During the period of the germination test, each solution was added as needed. The seeds remained for 21 days under a room temperature regime (27±2°C) and light cycle (8 hours of light/16 hours of darkness), when the percentages of germination were evaluated. All data were subjected to two-way ANOVA and these means were compared for significance by the LSD test at $p < 0.05$ using R software version 4.1.0 (R Core Team 2020).

Results

The germination percentage of cv. 'OKI-1' was highest at 12 minutes of soaking with 36N H_2SO_4, and declined with extended soaking, reaching a lethal duration after 24 minutes (Figure 4-2). Sulfuric acid scarification combined with priming in 0.2% KNO_3 and 100 ppm GA_3 substrates increased the germination percentage of cv. 'OKI-1' seeds. The highest germination percentage occurred when seeds were soaked in H_2SO_4 for 11, 12, and 14 minutes and sown in 0.2% KNO_3, 100 ppm GA_3, and DW substrates, respectively. Immersion of seeds in 36N H_2SO_4 for longer periods than specified resulted in a lower germination percentage. Almost no seeds germinated in 0.2% KNO_3 and 100 ppm GA_3 substrates when seeds were scarified for 24 minutes. The effective priming substrate to promote the high germination percentage of cv. 'OKI-1' grass seeds was 100 ppm GA_3, followed by 0.2% KNO_3, and DW, respectively. When seeds were scarified and germinated in 0.2% KNO_3 and 100 ppm GA_3 substrates, seeds stored at 5°C demonstrated a higher germination percentage than seeds stored at 25°C, in contrast to DW (Figure 4-2).

For seeds stored at 5°C, the germination percentage increased when the 36N H_2SO_4 soaking duration was increased to 8 minutes. This result was found in all substrates used to promote germination. When the soaking duration exceeded 8 minutes, the germination percentage gradually decreased. When the seeds were scarified for 24 minutes, the decrease would reach its lowest point. The priming substrate that promoted the highest germination percentage of cv. 'OKI-1' seeds was 100 ppm GA_3, followed by 0.2% KNO_3, and DW, respectively. However, there was not a significant difference in the average germination percentage of the cv. 'OKI-1' seeds sown in 0.2% KNO_3 and 100 ppm GA_3, but their averages were different from those sown in DW. Soaking for 8 and 16 minutes in 36N H_2SO_4 and sowing seeds in a 100 ppm GA_3 substrate results in the highest germination percentage (Table 4-3).

For seeds stored at 25°C, the germination percentage of seeds sown in DW and 100 ppm GA_3 increased when 36N H_2SO_4 soaking duration was increased to 8 minutes, and 4 minutes in 0.2% KNO_3. The germination percentage gradually decreased as the soaking duration was longer than 8 minutes. The decrease would reach its lowest point if the seeds were soaked in 36N H_2SO_4 for 24 minutes, the same as in the case of seeds stored at 5°C. The highest germination percentage of the cv. 'OKI-1' was achieved when seeds were scarified with 36N H_2SO_4 for 8 and 16 minutes, and then sown in 100 ppm GA_3 substrate, and seeds were scarified with 36N H_2SO_4 for 4 and 8 minutes, and then sown in 0.2% KNO_3. The average germination percentages did not show a significant difference between the types of germination promoting substrates (Table 4-4).

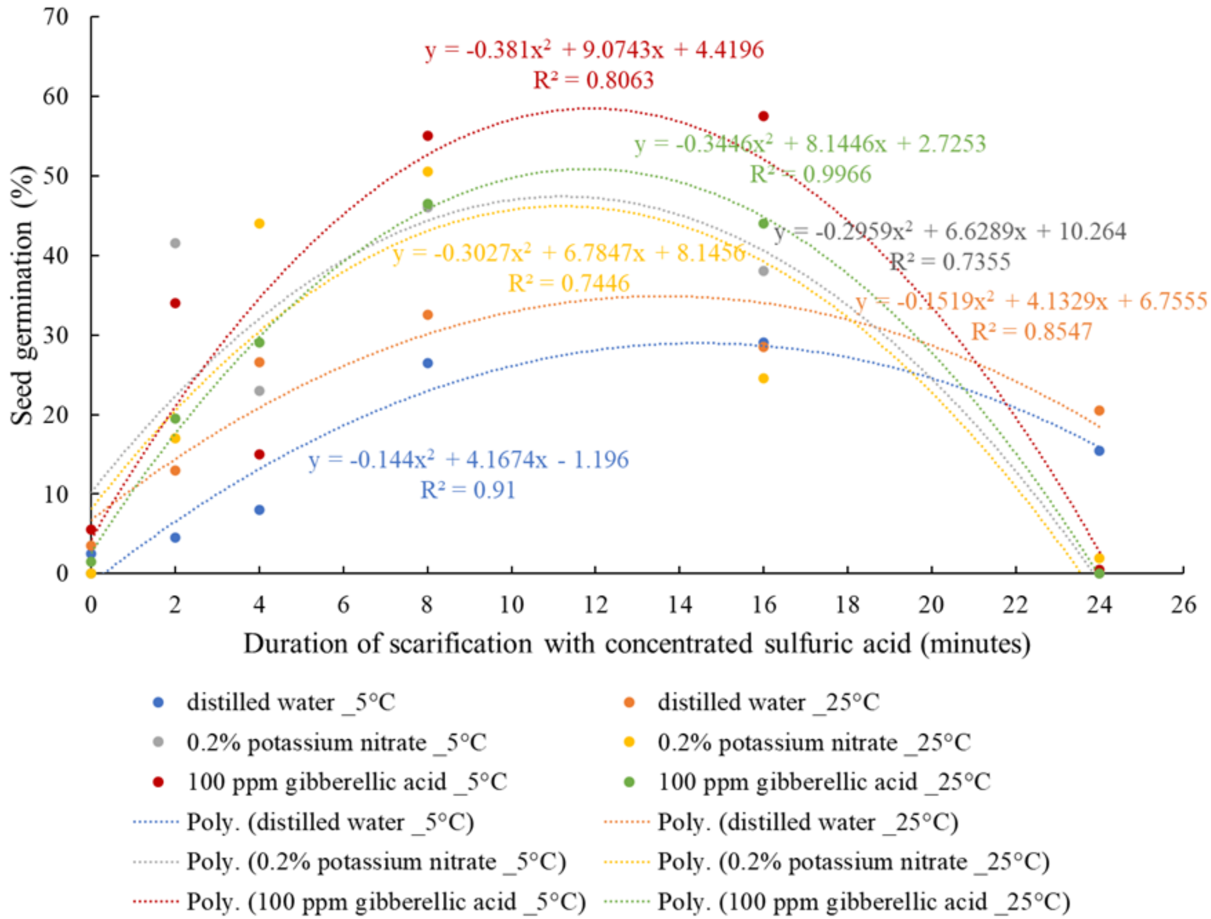

Figure 4-2 The relationship between the germination percentage of cv. 'OKI-1' seeds stored at two storage temperatures, scarified with concentrated sulfuric acid with varying soaking durations, and moistened with the three germination promoter solutions.

Table 4-3 The germination percentage of *Urochloa ruziziensis* cv. 'OKI-1' seeds was stored at 5°C, treated with concentrated sulfuric acid with varying scarification durations in various germination promoting solutions

Scarification duration (min.)	Substrate			Mean
	Distilled water	0.2% KNO_3	100 ppm GA_3	
0	2.50	0.00	5.50	2.67^{d}
2	4.50	41.50	34.00	26.67^{b}
4	8.00	23.00	15.00	15.33^{c}
8	26.50	46.00	55.00	42.50^{a}
16	29.50	38.00	57.50	41.50^{a}
24	15.50	0.00	0.50	5.33^{d}
Mean	14.33^{B}	24.75^{A}	27.92^{A}	

Note: Mean values followed by different superscript letters in the same row (capital letter) and column (small letter) indicate statistical differences ($p < 0.05$).

Table 4-4 The germination percentage of *Urochloa ruziziensis* cv. 'OKI-1' seeds was stored at 25°C, treated with concentrated sulfuric acid with varying scarification durations in various germination promoting solutions

Scarification duration (min.)	Substrate			Mean
	Distilled water	0.2% KNO_3	100 ppm GA_3	
0	3.50	0.00	1.50	1.67^{d}
2	13.00	17.00	19.50	16.50^{c}
4	26.00	44.00	29.00	33.00^{b}
8	32.50	50.50	46.50	43.17^{a}
16	28.50	24.50	44.00	32.33^{b}
24	20.50	2.00	0.00	7.50^{d}
Mean	20.67^{A}	23.00^{A}	23.42^{A}	

Note: Mean values followed by different superscript letters in the same row (capital letter) and column (small letter) indicate statistical differences ($p < 0.05$).

Discussion

The results found in this study indicate that all the evaluated scarification durations with concentrated (36N) H_2SO_4, independent of the storage temperature, increased the germination percentage of the cv. 'OKI-1' seeds in comparison with the un-scarified seeds. Seed scarification with 36N H_2SO_4 was an efficient treatment for breaking dormancy of the cv. 'OKI-1' seeds. This finding is consistent with previous studies on *Urochloa* grass cv. 'Mulato II' (Hare et al., 2018; 2008) and *U. brizantha* cv. BRS Piatã seeds (Romani et al., 2016), *U. brizantha* and *Panicum maximum* (Usberti & Martins, 2007), which found that acid-scarified seeds could be achieved to a higher germination percentage. All the aforementioned studies revealed that scarification of seeds with 36N H_2SO_4 for 10 minutes was effective. This duration of seed scarification with 36N H_2SO_4 corresponded with the present study, which recommended that 10–12 minutes of seed scarification with 36N H_2SO_4 be suitable for seed dormancy breaking to achieve the highest germination percentage. However, the highest germination percentage was achieved when combined with the priming seeds with 0.2% KNO_3 or 100 ppm GA_3. Scarified seeds with 36N H_2SO_4 for 8 minutes when sown in 0.2% KNO_3 and 100 ppm GA_3 substrates could promote higher germination than sown in DW substrate. When seed germination percentages were compared at the same scarification duration, seeds stored at 5°C had a higher germination percentage than seeds stored at 25°C. This result was observed in seeds germinated in 0.2% KNO_3 and 100 ppm GA_3, while seeds germinated in DW achieved the contrary result. Seed storage conditions may have influenced this result. Hare et al. (2013) reported that seed germination of cv. 'Mulato II' and *Panicum maximum* deteriorated rapidly under ordinary ambient conditions in Thailand when seeds were stored for more than 1 year. Seeds of cv. 'Mulato II' not scarified in acid and stored in the cool room maintained very low germination (0–23%), but once scarified with acid, germination increased to 75–88%.

The lowest germination percentage was observed in un-scarified seeds, and seeds were scarified with 36N H_2SO_4 for 24 minutes. Although seed can be treated with 36N H_2SO_4 to break dormancy, this is dangerous to the seed if left too long in acid or not washed thoroughly. This is confirmed by the present study, which found almost no seeds germinated if seeds were soaked in 36N H_2SO_4 for 24 minutes.

The findings of the study indicated that H_2SO_4 scarification could be effective for breaking seed dormancy of cv. 'OKI-1', the same as the other commercial *Urochloa* cultivars. The duration of soaking cv. 'OKI-1' seeds in 36N H_2SO_4 for 10–12 minutes was optimum for the maximum percentage of germination to be obtained. However, although soaking seeds in 36N H_2SO_4 has been widely used and is effective in breaking the dormancy of *Urochloa* seeds, this treatment raises inconveniences relating to operator safety during application. Moreover, refusal of residues from this chemical treatment may become harmful to the environment. Consequently, the other methods for breaking the dormancy of cv. 'OKI-1' were required for further study.

As observed in the present study, the combination treatment for breaking dormancy of cv. 'OKI-1' seeds by H_2SO_4 scarification and priming by moistened seeds with 100 GA_3 solution demonstrated the highest effectiveness in germination percentage. This is confirmed by Vieira et al. (1999), who reported that the physiological dormancy of *U. brizantha* seeds was partially released by low pH solutions and by gibberellic acid. The combination of low pH solution with gibberellic acid showed an additive effect in the promotion of the germination of dormant seeds. The low pH could have facilitated the penetration of the gibberellins through the membrane lipid constituent since they were in a protonated form. Alternatively, an increase in hydrogen ion concentration could have caused changes in the cell wall, allowing for growth, a phenomenon known as the acid effect. In dormant seeds of *Urochloa*, it is possible that another mechanism was active since pH 2.0 or gibberellic acid alone was able to cause dormancy break and, as mentioned, the simultaneous action of both was additive. The germination of dormant *Urochloa* seeds was enhanced by the addition of gibberellic acid supplied in solution at pH 2.0. However, the effectiveness of the dormancy breaking method usually varies among species and even cultivars or accessions. It is necessary to understand the mechanisms involved in treated species to choose the optimal method (Wang & Hu, 2013). The breaking dormancy treatments that were tested in this study could be used in a laboratory to evaluate the germination of seeds, which may be useful for trading. However, practical dormancy-breaking treatments for field work will be required.

In the case of ruzigrass (*U. ruziziensis*), the period of seed dormancy is normally 4 to 6 months but can extend to 18 months if the seed is stored at a low temperature (Devahuti & Sirisompan, 1985). Ruzigrass seed needs to be stored for at least 6 months after harvest to overcome dormancy, but quality deteriorates if the seed is kept too long at high temperatures and high humidity. Because most *Urochloa* spp. seeds in Thailand are harvested from November to December, and because the next planting season begins in May of the following year, the harvested seeds remain dormant. As a result, if farmers want to use cv. 'OKI-1' seeds, they must use practical and effective methods to release or break dormancy.

Conclusion

Scarifying seed with concentrated H_2SO_4 is an efficient method to overcome seed coat dormancy. The combination of H_2SO_4 scarification for 10–12 minutes and priming with 100 ppm GA_3 as a germination promoting substrate promoted an increased germination percentage of the new *Urochloa* spp. (cv. 'OKI-1') seeds, which seeds were both stored at temperatures of 5°C and 25°C.

CHAPTER 5

General discussion, conclusion, and recommendation

5.1 General discussion and conclusion

The overall objective of this thesis was to investigate the seed yield and quality of two novel cultivars of *Urochloa* spp., namely cv. 'OKI-1' and cv. 'Br-203' in Northern Thailand. The study aimed to assess the feasibility status of the two new cultivars of seeds produced in infertile acid soil areas under six-month dry season conditions and thereafter evaluate how seed yield and quality are influenced by plant spacing, nitrogen fertilizer level, closing cut date, and method of harvest. The first experiment in Chapter 2 is aimed at seed yield assessment compared with the other commercial *Urochloa* cultivars, to evaluate whether it is profitable to invest in a new *Urochloa* spp. establishment for seed production under the weather conditions in Northern Thailand. The other two experiments in Chapter 2 aimed to evaluate the appropriate cultivation practices, i.e., plant spacing, closing cut date, and nitrogen fertilizer application for maximized seed production of the two grass cultivars. Furthermore, the appropriate harvesting method that could maximize seed yields of cv. 'OKI-1' was evaluated in Chapter 3, and how to improve harvested seed germination by chemical treatments was assessed in Chapter 4. The main findings of these studies may therefore be summarized and concluded as follows:

1) Even though both new cultivars demonstrated high forage yield potential, drought tolerance, and the ability to grow in low nutrient quality soil, seed yields were disappointing. Both candidate cultivars have favorable characteristics similar to several *Urochloa* grasses planted in Thailand, such as pest resistance, drought intolerance, ability to grow in infertile acid soil, and producing high forage yield. Based on the present study, seed yields of cv. 'OKI-1' were similar to those of cv. 'Kennedy' and cv. 'Mulato II', which are commercial *Urochloa* widely used for seed production in Thailand. However, the produced seed yields were less than the seed yields of commercial *Urochloa* in some countries, which produce seeds for

international seed trading. Therefore, the seed production potential of cv. ‘OKI-1’ in Northern Thailand is still not competitive with the other commercial *Urochloa* seeds being produced in other parts of the globe, such as Brazil and Australia. With the seed yield of cv. ‘OKI-1’, which is similar to the average seed yields in terms of quantity and quality of cv. ‘Kennedy’ and cv. ‘Mulato II’ planted in Thailand, but it has higher digestibility than those of two cultivars (Thaikua, 2015), it is possible to produce seeds commercially and can be acceptable to farmers who feed cattle and seed growers. The cv. ‘Br-203’ is notable for its forage yield, but the available seed yields were extremely low. As a result, they cannot be grown as a forage crop to produce commercial grass seed. TSY, PSY, and PGSY of cv. ‘Br-203’ were similar to cv. ‘Basilisk’, though cv. ‘Br-203’ has numerous seeds and seems to have more seeds than cv. ‘Basilisk’ or even cv. ‘OKI-1’. This was caused by the cv. ‘Br-203’, which has a lot of shriveled seeds. Despite superior dry season forage production, broader adaptability, and persistence of cv. ‘Basilisk’, it has not been accepted by seed growers in Thailand because of the difficulty of producing seed. Because the cv. ‘Basilisk’ produces seed over 2–3 months in the wet season, during a period of high precipitation, whereas the other *Urochloa* cultivars produce seed in a 1-month period, with very little rainfall at the beginning of the dry season. Considering seed quality in terms of germination percentage, the minimum standard of 60% germination, viability, or emergence, required for the commercialization of forage seeds, should be maintained to ensure the ideal forage population in the establishment of the crop (Avelino et al., 2019). In this experiment, for most grass cultivars, except for the cv. ‘Mulato II’, the germination percentage value was more than the recommended minimum standard. The cv. ‘OKI-1’ seeds had the highest germination percentage (83.3%) on a two-year average. As a result, commercialization of cv. ‘OKI-1’ seed is highly likely if seed yields can be increased further.

2) The preliminary studies at several sites with different climate and soil conditions in Thailand indicated that both cv. 'OKI-1' and cv. 'Br-203' were feasible to grow for forage in North and Northeast Thailand. Proper cultivation practice management is one of the most important factors affecting economically viable seed production. The present studies were thus subsequently initiated to develop agronomic information for seed growers interested in improving their field operations and increasing opportunities for seed trading. Knowledge of the appropriate plant spacing is a fundamental requirement for any grass seed crop, as plant spacing normally has a strong influence on the yield and longevity of the stand. Seed yield of cv. 'OKI-1' and cv. 'Br-203' can be optimized by establishing an initial plant spacing of 100 × 100 cm and 75 × 100 cm, respectively. In practice, excellent stands have been achieved with 136.46 and 79.59 kg/ha of PSY. Based on this experiment, the seed yields were very low compared with the average of the other commercial *Urochloa* seed yields that can be produced in Thailand, i.e., 250–600 kg/ha in cv. 'Mulato II' (Hare, 2021) and 300–500 kg/ha in cv. 'Kennedy' (Phaikaew et al., 1993). Moreover, the PSY of this experiment was less than two times that of other experiments in this thesis study. It may be caused by the texture and properties of the soils at the experimental site being a sandy soil with poor nutrient quality. Soil texture and structure greatly influence water infiltration, permeability, and water-holding capacity. Soil moisture limits forage seed production potential. The capacity of soil to hold water depends upon the size of soil particles. The sandy soil at this site has a larger particle size than the other experimental sites, so it has a very low capacity for holding water. Furthermore, the soils at the other experimental sites have more clay soil than at this trial site. Because the clay soil has small particles, it has a larger surface area and thus retains more water.

For most species, growing grass seed crops in rows rather than swards is recommended in the first year, and for some species, continued row culture is recommended. Row culture may become mandatory as a result of seed certification schemes. Row planting with the recommended plant spacing should be considered for the two new candidate *Urochloa* cultivars. Because the following benefits are claimed:

i) The seeding rate is reduced, allowing a larger multiplication area to be sown from a smaller amount of breeder's or basic seed.

ii) Off-types can be identified and rogued more reliably, and crop inspection is made easier.

iii) Weed control and other management practices such as cutting, applying fertilizer, and harvesting are facilitated.

iv) Moisture and nutrients are distributed more evenly.

v) Appropriate plant spacing may provide a better light environment for the flowering shoot. This assumes that plant density will also be adjusted to reduce inter-plant competition.

The optimal time of closing cut can have an impact on seed production. For the two new candidate *Urochloa* cultivars, the CCD in late-June to early-July and early-July to mid-July is recommended for cv. 'OKI-1' and cv. 'Br-203', respectively. Seed yield can be decreased if the closing cut was too early or there was a delay from the recommendation. The seed yields in cv. 'OKI-1' and cv. 'Br-203' were non-significant in response to the different N-rates in the fertilization timing in the current study. It might be caused by very low initial soil nitrogen. All the N-rates that were used may not be sufficient to make the seed yield different. However, nitrogen fertilizer must be applied after the closing cut to promote tiller multiplication. It should be noted that soil moisture at the time of applying nitrogen fertilizer, especially in a urea form, is an important factor affecting the amount of useable nitrogen by a plant. Alternatively, split nitrogen fertilization may contribute towards satisfying the plant nitrogen demand through soil supply, thereby improving nitrogen use efficiency and ultimately improving seed yield. Moreover, the effect of split nitrogen fertilization on seed yield under water-saving irrigation conditions warrants further investigation.

3) Harvesting management is a crucial step to achieving the seed in terms of quantity and quality. Aside from choosing the proper time of harvest, choosing the appropriate method for harvesting is an important management decision. Different seed harvesting methods resulted in varying yields and quality of grass seed. The quality of the seeds depends on the time of harvest and the method of harvest. Based on long-term experience of grass seed production from many studies in Thailand, developed harvesting methods were applied suitable for grass species. Hand-harvesting is still necessary for small-holder farmers. The study in Chapter 3 recommends that allowing ripe seeds to fall into a nylon net sheet stretched as a receptacle positioned beneath the seedheads, 1 m above the ground, and collecting them every five days is suitable for manual seed harvesting in all two new *Urochloa* cultivars. Aside from obtaining a higher seed yield, the method is also easier to handle seed collection, less time-consuming, and minimizes labor costs. Despite the fact that this method is at risk of being destroyed by pests (i.e., insects, birds, and rodents), strong winds, and heavy rain, frequent seed collecting is likely to mitigate the problem. Additionally, the recommended method could avoid seedhead damage caused by covering them with a nylon-net bag and directly knocking the seedheads. It can be used substantially the same way as the conventional methods used by small-holder farmers in Thailand.

4) The seed-covering part of two novel *Urochloa* spp. (cv. ‘OKI-1’ and cv. ‘Br-203’) does not impede water absorption, which leads to being the cause of seed dormancy as well as obstructing germination. Seed dormancy breaking and germination promotion were better achieved when seeds were soaked in concentrated H_2SO_4 for 10–12 minutes before being primed with 100 ppm GA_3 used as substrate. This treatment could be used for laboratory seed

testing and seed trading preparation, but it may not be suitable for preparing seed used in field work by smallholder farmers.

5.2 Recommendations

Based on the findings obtained from this study, it is recommended that:

1) More research on the seed production of two new cultivars in different locations and weather conditions in Thailand or other countries is needed to gain a better understanding of the grass seed production of both grass cultivars.

2) Although the plant spacing had no effect on seed yield, both new cultivars tended to produce the highest seed yield with good quality at an optimum plant spacing, which for cv. ‘OKI-1’ and cv. ‘Br-203’ was 100 × 100 cm and 75 × 100 cm, respectively. Aside from the recommended optimum plant spacing, adequate fertilizer and appropriate environmental factors may increase seed yield. However, proper plant spacing was also determined by the soil quality and irrigation. As a result, before establishing a field and determining plant spacing, the aforementioned factors should be considered.

3) Before planting, soil fertility should be adjusted using appropriate nitrogen and phosphorus rates. However, the use of green manure or compost as a partial substitute for fertilizer could be a strategy to minimize the cost of production. Keep in mind that contaminated weed seeds in manure may necessitate post-planting weed control.

4) To maximize seed yields, seed growers should prepare effective plans for repelling birds and rodents during the dry period, which is heavily emphasized from flowering to seed maturity.

5) It is recommended that a long-term study be conducted on how to manage and sustain the basal tillering of plant stands for high yields and quality seeds.

6) To maximize seed yields, irrigation may be required, particularly during dry spells. Building ridges and furrows may be required when considering soil textures and structures for effective use of water by plants.

7) For practical use, more research is needed to improve seed germination of cv. ‘OKI-1’ by breaking dormancy and promoting germination through a variety of methods.

Summary

Urochloa is a genus of forage plants in the grass family that are native to tropical and subtropical grassland regions of Asia, including Okinawa, Africa, Australia, and South America. The success of new forage species depends on their palatability, ability to produce high dry matter, nutritional quality, yield stability, pest and disease resistance, and high capacity to produce viable seeds. Considering latitude and climatic conditions, northern Thailand was chosen for *Urochloa* spp. seed production practices. In this study, the seed production of *Urochloa* spp. under different cultivation practices for maximizing seed yield was examined. The summary of the test results is as follows:

1) As a result of comparative seed production of the five *Urochloa* cultivars, the novel cultivars ‘OKI-1’ and ‘Br-203’ produced sufficient inflorescences, racemes, and spikelets which had the potential for useful seed yields compared with commercially available cultivars such as *U. decumbens* cv. ‘Basilisk’, *U. ruziziensis* cv. ‘Kennedy’, and hybrid *Urochloa* cv. ‘Mulato II’.

2) Two field trials were conducted to determine the optimal plant spacing for seed production of two novel *Urochloa* cultivars, cv. ‘OKI-1’ and cv. ‘Br-203’. The cv. ‘OKI-1’ and cv. ‘Br-203’ tended to have higher pure seed yields (PSY) at plant spacings of 100 × 100 cm (136.5 kg/ha) and 75 × 100 cm (79.6 kg/ha), respectively. Both cultivars showed similar trends in PSY, filled seed percentage, and a thousand seed weight.

3) Optimum cultivation practices were evaluated to maximize seed production for two novel *Urochloa* cultivars, including a closing cut date (CCD) and nitrogen application rate (N-rate). As a result, the highest seed yields in cv. ‘OKI-1’ and cv. ‘Br-203’ were obtained when the CCD was early-July and early-July to mid-July, respectively, regardless of N-rate within 0–150 kgN/ha.

4) In the two *Urochloa* cultivars, allowing ripe seeds to fall into a nylon net sheet stretched as a receptacle beneath the seedheads, 1 m above the ground, and collecting them every five days or more frequently is suitable for hand-harvesting of seed.

5) As a result of breaking dormancy and an effective method to promote germination rate, the following germination-promotion process was most suitable. The combination of H_2SO_4 scarification for 10–12 minutes and priming with 100 ppm GA_3 as a germination promoting substrate promoted the germination percentage of the new *Urochloa* spp. (cv. 'OKI-1') seeds when seeds were stored at temperatures of 5°C and 25°C.

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要約

ウロクロア属は、沖縄を含む熱帯・亜熱帯のアジア、アフリカ、豪州および南米地域の草地群落を構成するイネ科の主要な１属である。新規草種・品種開発の鍵となる形質は、家畜の嗜好性、乾物生産性、飼料品質、安定生産性および病害虫抵抗性に優れ、並びに、発芽能の高い完熟種子を生産できることである。ウロクロア属草種・品種の種子生産の栽培試験は、緯度や気象条件を考慮し、タイ国北部地域が選定された。

本研究は異なる栽培条件下でのウロクロア属草種・品種の種子生産収量を高める栽培学的追究と発芽促進技術の開発を行ったものである。得られた結果は以下の通りである。

1）ウロクロア属草種・品種のうちで、導入新品種 cv. ‘OKI-1’ と cv. ‘Br-203’の種子生産性を、市販の普及品種である *U. decumbens* cv. ‘Basilisk’、*U. ruziziensis* cv. ‘Kennedy‘、およびハイブリッド品種の Mulato II と比較したところ、生産性の指標となる開花数、総状花序数および小穂数では、草種・品種間に一定の傾向は認められず、成熟種子収量では有意な差は認められなかった。

2）ウロクロア属の新品種 cv. ‘OKI-1’ と cv. ‘Br-203’ を対象に、種子生産性を高める栽植密度の検討を２年連続で行った。その結果、cv. ‘OKI-1’では 100×100 cm、cv. ‘Br-203’では 75×100 cm で最も高く、成熟種子収量はそれぞれ、136.5 kg/ha と 79.6 kg/ha であった。また、１年目の生産量が高く、成熟種子の着生率や千粒重が高いことが要因であった。

3）多年生牧草種であるウロクロア属草種の種子生産の重要な要因となる収穫前の直近刈取り時期（CCD）と窒素施肥量(N-rate)について、cv. ‘OKI-1’と cv. ‘Br-203’を対象として栽培評価試験を行った。その結果、‘OKI-1’ と ‘Br-203’の最大種子収量が得られた CCD は、それぞれ 7 月初期、7 月初期〜中期であった。しかしながら、0–150 kg N/ha の N-rate では、いずれの CCD でも種子生産に影響はほとんど認められなかった。

4）新２品種を栽培し、有効な成熟種子の収穫方法について実証試験を実施した。その結果、ナイロン・ネット・シートを地面から約 1m に設置し、落下した成熟種子を 5 日毎に集める方法によって最も多い収穫量を得られた。

5）収穫後のウロクロア属の草種・品種は休眠種子が多い。収穫後の‘OKI-1’を供試して休眠覚醒のための事前処理を行い、発芽率を促進する方法を検討した。その結果、5℃または 25℃で保蔵した場合でも、10-12 分間の濃硫酸で種皮処理を行い、100 ppm のジベレリン酸溶液に浸すことが休眠覚醒をもたらし、発芽率を高める方法として最も有効であることが明らかとなった。