Original Research Article||

Variability through crossing and subsequent selection towards a commercial cultivar development in summer flower *Hypericum androsaemum* L.

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Abstract

Hypericum and rosaemum L., a popular summer flowering plant, faces significant challenges in commercial cultivation due to susceptibility to leaf rust diseases. These diseases affect marketability, forcing reliance on fungicides that increase costs and pose environmental concerns. This study aimed to enhance the commercial potential of *H. androsaemum* by introducing rust resistance gene through crossing a rust-susceptible commercial cultivar with a rust-resistant weedy genotype. The crossing was conducted at Milko Flower Farm, central Ethiopia, in 2021, resulting in viable F1 progenies. From the F1 population, five progenies were selected based on their desirable traits, such as tall stems, deep green rust-free leaves, and large deep red berries. Subsequent trials in 2022 using a completely randomized block design revealed that Progeny One exhibited the most favorable traits, including uniformity, distinctiveness, and reproducibility compared to the commercial cultivar used as a parent in crossing. This progeny was named H. androsaemum 'Ruby Excess,' registered and now grown commercially for export. The study demonstrates that breeding programs integrating rust resistance with ornamental traits can reduce fungicide use, enhance marketability, and mitigate environmental impacts. Furthermore, the methodology employed in this study could be adapted to introduce rust resistance into other commercial cultivars of H. androsaemum. However, the viability of F1 seeds and proximity of weedy populations to flower farms highlight the need for measures to prevent unintended spread and potential invasiveness. Therefore, implementing measures to prevent unintended spread is essential.

Key words: Crossing, Hypericum androsaemum, New cultivar, Ruby Excess, Rust resistance, Weedy genotype.

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INTRODUCTION

Floriculture is a vital component of global agricultural trade, with Ethiopia emerging as Africa's second-largest flower exporter, following Kenya (Dirriba and Mihretu, 2024). The sector contributes significantly to the national economy by creating employment opportunities and generating foreign exchange. Flowers have become Ethiopia's second most important export commodity after coffee, representing 14.1% of total export earnings. floriculture accounts Furthermore. for an impressive 80% of Ethiopia's horticultural revenue, showcasing its role as a key driver of agricultural

development (Abas and Dame, 2015; Dirriba and Mihretu, 2024). Among the various flower species cultivated in Ethiopia, *H. androsaemum*, commonly known as tutsan, is particularly notable for its dual value: ornamental appeal and potential pharmacological benefits (Dias, 2003; Chavez and Lyrene, 2009).

H. androsaemum is a tetraploid species (2n = 40) well-known for its bright yellow blooms and striking berries, enhancing its appeal in horticultural markets (Dias, 2003; Chung et al., 2010). In contrast, other taxa in different sections

exhibit a chromosome number of 2n = 20 (Robson and Adams, 1968). However, inheritance studies conducted by Olsen et al. (2006) demonstrated that the segregation patterns for foliage traits in H. androsaemum align with a diploid model. It is part of the Hypericum genus, which includes approximately 400 species of trees, shrubs, and herbs. The features that set H. androsaemum apart from other species in the Androsaemum section are its shorter styles and unique black berries, which stay soft even after ripening (Huxley et al., 1992). The species originates from temperate areas of Europe and Western Asia (Bailey, 1949) but has expanded beyond its native habitat because it is grown for decorative purposes. It has been naturalized in areas like Australia, New Zealand, and Chile through horticultural trade (Heenan, 2008).

In Australia, New Zealand, and the northwestern United States, it is considered an invasive species (Weber, 2003; Olsen et al., 2006). In Ethiopia, H. androsaemum was introduced during the expansion of the cut flower industry. While its cultivation has boosted the local horticultural trade, it has also raised ecological concerns. Weedy populations of the species are often found growing near flower farms, where they are cultivated for commercial purposes. Observations suggest that these weedy populations could potentially become invasive, as seen in other parts of the world. For instance, in Australia, H. androsaemum has been classified as an invasive species, with studies showing that its increasing abundance negatively impacts native species richness (Carey, 2007).

Hypericum androsaemum (tutsan) reproduces both insect pollination, sexually, through and vegetatively, enhancing its adaptability for natural propagation and breeding programs. The backcross breeding method involves mating a hybrid offspring with a recurrent parent lacking a desired resistance gene. This gradually incorporates the rust resistance gene from the donor parent into the recurrent parent, which retains its ornamental traits despite rust susceptibility. This strategy effectively transfers beneficial genes while preserving aesthetic qualities. The primary production regions for Hypericum androsaemum in Ethiopia, including Wolkite, Mehal Amba, Woliso, Holeta, Sebeta, and

Ginchi, face significant challenges from leaf rust disease caused by the fungal pathogen Melampsora hypericorum. Melampsora hypericorum is an orange rust fungus that attacks Hypericum species, particularly Tutsan (Hypericum androsaemum). It forms yellow aecia on the leaf undersides, resulting in corresponding yellow or reddish patches on the upper surfaces. Rust infections render plants unmarketable, forcing growers to rely heavily on fungicides, which increase production costs and pose environmental risks. Identified in Australia in 1991, M. hypericorum has caused significant declines in H. androsaemum populations in certain areas, although some populations exhibit natural resistance. Breeding programs that combine rust resistance with desirable ornamental traits offer a sustainable and cost-effective alternative to fungicides, reducing reliance on chemical inputs while maintaining plant quality and market value. Dias (2003) and Carey (2007) highlight the potential of such breeding strategies, while Wise et al. (2004) emphasize the broader economic and regulatory challenges posed by ornamental rusts, including reduced plant value, unmarketable products, and increased costs. These studies stress the urgent need for research into rust-resistant cultivars and advanced diagnostic tools for effective disease management.

The success of any crop improvement program depends heavily on the availability of genetic variability and heritability of the traits of interest. In the case of *H. androsaemum*, genetic variability can be harnessed through conventional breeding techniques such as germplasm introduction, plant selection, and hybridization, as well as through modern biotechnological tools (Bekele and Gedebo, 2020; Belay et al., 2024). These methods enable the introduction of desirable traits, such as rust resistance, larger berries, deep green foliage, and vibrant colors, into commercial cultivars. Crossing genotypes with complementary traits generates genetic variability, which can then be exploited to develop superior cultivars through subsequent selection and propagation. Long before the advent of molecular genetics, Biffen (1905) demonstrated that rust resistance in some crops followed Mendel's laws of inheritance. This foundational work laid the groundwork for modern rust-resistance breeding efforts. Leus (2018)

discusses breeding strategies for disease resistance in ornamental plants, highlighting the importance of reducing chemical treatments and improving plant health, serving as a model for similar work in *H. androsaemum*.

Trueblood et al. (2010) conducted cross-breeding of H. androsaemum to develop triploid clones with traits such as attractive foliage and reduced invasiveness. Although these clones exhibited the desired qualities, they were infertile. In contrast, other forms of H. androsaemum were fertile, highlighting the challenge of breeding non-invasive cultivars that also maintain fertility. Inspired by this, we observed healthy, rust-free weedy Hypericum genotypes growing naturally near Milko Flower Farm in Central Ethiopia. These weedy plants exhibited favorable traits, such as deep red berries and rust-free leaves, but had some limitations, like smaller berries and lighter green leaves. On the other hand, the commercial cultivar featured larger berries and green leaves, but had undesirable traits like light red berries and rustprone leaves. The goal of this study was to develop new cultivars that combine the rust resistance of weedy genotypes with the ornamental appeal of commercial varieties.

MATERIALS AND METHODS

The Study Area

The study was conducted at Milko Flower Farm in Central Ethiopia, located 136 kilometers from Addis Ababa, within the Gurage Zone, specifically in Gedebano Gutazer Welene District, Desa kebele (14° 56' 52.33" N, 39° 9' 15.72" E). The farm is approximately six kilometers from Mehal Amba town and sits at an altitude of 2,300 meters above sea level. The research took place in an open field rather than in a greenhouse. The soil type is clay loam, characterized by a high water-holding capacity. The area experiences an annual rainfall of 151 mm, with a distinct seasonal pattern. The peak rainfall occurs during August and September, coinciding with the region's main rainy season. In contrast, the driest period is observed between December and February, with minimal precipitation. The temperature in the area also shows moderate variation, with a recorded minimum temperature of 9°C and a maximum temperature of 24°C, reflecting a relatively mild climate conducive to agricultural and ecological activities. These climatic characteristics play a significant role in determining the area's vegetation, water availability, and agricultural potential.

Plant Materials

The study began with the identification of two parent genotypes of Hypericum androsaemum. Milko Flower Farm provided the first parent genotype *Hypericum* and rosaemum 'Shiney Romance' (P1) a commercial cultivar that was rust susceptible, while having lovely decorative qualities including large, light red berries and deep green leaves. The second parent genotype (P2) of the same species was obtained growing weedy at the farm edges of Milko Flower Farm. Notably, the plants found at the farm's edges produced rust free light green leaves and small sized red berries. It is believed that these plants may have originated from other Hypericum cultivars that grew at the farm in previous years.

One hundred plants of each parent were established as a mother stock from stem cuttings in 2020. The two parent stocks were established on two separate beds of 10m² (1m width x 10m length) each with 50cm spacing between beds. The genotypes were planted with a spacing of 50 cm between rows and 20 cm between plants within a row. The plant nutrients commonly used for commercial cultivars that thrive in the same nutrient-depleted plots for multiple years were considered not applicable to the current study. Since this study was conducted on plots where Hypericum has not previously been grown, and the soil is not depleted, only standard fertilizer nutrients were utilized. Therefore, Nitrogen fertilizer in the form of urea 100 kg/ha was side dressed in split immediately after planting and 30 days after planting. The blended fertilizer NPSB 50 Kg/ha was side dressed at planting. The plots were watered twice a day, in the morning and in the afternoon, to a field capacity.

Breeding Procedures

The breeding procedures involved synchronizing flowering stages of parent plants, inducing flower initiation, cross-pollination, and selecting progeny. These steps are outlined as follows.

Synchronizing Flowering

In 2021 the stems of the two plots were cut back uniformly at height of 10 cm above ground after 60 days from planting. Twenty days after cutback, a new flush began to emerge, and forty days after the cutback, the stems were thinned back to one stem per plant. Once the plants grew to a height of 40 cm, we provided additional light using a compact fluorescent (CFL) bulb with an intensity of 150 lux and a wavelength of 610-700 nm. This bulb was positioned three meters above the canopy of the plants and was used for six hours each day, from 6 PM to midnight, for a duration of 30 days. Since Hypericum is a long-day plant, the extra light was needed for achieving uniform flower induction.

Cross Pollination

At flowering, before anthesis, the anthers of 25 female parent plants (P1) were removed to emasculate the flower, and they remained covered with a protective bag to prevent contamination or unintended pollination. The anthers of the male parent (P2), however, were not emasculated. At anthesis, the mature anthers from P2, which contained pollen grains, were carefully collected and used to dust the stigma of P1. The stigma of P1 was then kept covered for 15 days to avoid contamination until fruit formation was completed, after which the protective cover was removed. When the berries reached maturity and turned black, the seeds from P1 were collected.

Progeny Selection

On first of January 2021, F1 seeds were sown in a bed measuring 10 m^2 (10 m in length and 1 m in width). The spacing between rows was set at 30 cm, and within each row, seeds were planted 20 cm apart, with two seeds per hill. When the seedlings grew to a height of 40 cm additional light was given to initiate flowering using compact fluorescent (CFL) bulb with an intensity of 150 lux and a wavelength of 610-700 nm for six hours each day, from 6 PM to midnight, for a duration of 30 days.

At flowering, the plants produced berries that varied in size and color intensity, and produced leaves varied in greenness, as well as resistance to rust. Since there was a noticeable incidence of rust at the farm, additional rust inoculation was not necessary. Among the plants evaluated, those that produced relatively larger and deep red berries and deep green leaves, with no rust spots, were selected. A total of 300 progenies were assessed, and five top-ranking progenies were chosen. These five F1 progenies were considered to be five potential cultivars. On first of July 2021 these five progenies and two parent genotypes were planted on seven separate plots each $5m^2$ (5m length and 1m width) using stem cuttings as a planting material to establish mother stock.

Experimental Design

On May 1, 2022, stem cuttings from five progenies and two parental controls (P1 and P2) were planted in seven separate beds, each measuring 5 m² (5 m in length and 1 m in width), with three replications. The experimental design utilized was а Randomized Complete Block Design (RCBD). The spacing between rows was set at 30 cm, while the spacing between plants within each row was 20 cm, resulting in three rows in each bed. At the height of 30 cm all the plants were cut back to the height of 10 cm from ground, to put all the progenies at the same starting point for subsequent evaluation. Several stems sprout from the cut point. These were thinned back to one stem per plant. Sixty days after cutback, additional light was provided as usual to achieve uniform flower induction. The same rates of standard fertilizers were applied as described under the Planting materials section.

The field evaluation was carried out from May to the end of November, during which the weather in the experimental area was predominantly rainy. The humid and wet conditions of this season promote fungal growth, making it an ideal time to select plants that are resistant to rust.

Data Collection

Data collection was conducted by experienced supervisors at Milko Flower Farm. The supervisors' skill was valuable as the size and color of the berries, along with the color were assessed through visual evaluation. Berry size and color, as well as leaf color, were determined at the stage of full berry growth, which coincides with the time when flowers are typically harvested for sale. However, the incidence of leaf rust was evaluated throughout the growth period. The progenies with their parents were assessed based on several characteristics: berry color (deep red and light red), relative berry size (small and large), leaf color (light green and deep green), stem length was measured for 10 plants per plot at flowering and resistance to rust was recorded as the percent leaf cover by the rust.

Data Analysis

The analysis of variance was done for days to flowering, stem length and leaf rust intensity (percent of leaf coverage by the rust) using MINITAB version 19. The mean separation was conducted using the Least Significant Difference (LSD) method at a significance level of 5%.

RESULTS AND DISCUSSION

Seed Viability and Invasiveness

In this study, cross-pollination between a commercial cultivar and a weedy genotype of Hypericum androsaemum produced viable seeds in the F1 generation, demonstrating the species' invasive potential. The presence of weedy plants at farm edges further underscores its ecological significance as a weed, a characteristic also observed in regions like New Zealand and Australia (Heenan, 2008). In contrast, Trueblood et al. (2010) found that triploid clones of *H. androsaemum* lack female fertility, suggesting their non-invasive nature. This duality highlights the importance of controlled production and management to balance the ornamental value of the plant with its invasive risks. In Ethiopia, strategies such as composting plant materials from flower farms and utilizing triploid clones could help minimize the spread of H. androsaemum. Additionally, harvesting before seed maturity, when berries are fully developed but not yet dispersed, could further reduce the plant's invasiveness.

Resistance to Leaf Rust

In the current study, the progenies of *Tutsan* exhibited significantly varying responses to rust fungus (P < 0.001, Table 4), with Progeny One showing rust-free leaves (Table 5). This aligns with Casonato et al. (1999), who found that some *Tutsan* populations have natural resistance to rust. However, McLaren et al. (1997) observed that *Melampsora hypericorum* caused varying damage to *Tutsan* and was even used as a biological control

to manage its invasiveness. Previous research has identified other strains of the rust fungus in Australia (Baker, 1955; Whatman, 1967), but these strains were ineffective in controlling *Tutsan* (Groenteman, 2009). The reasons for these discrepancies are not well understood and may involve factors such as environmental conditions, genetic susceptibility, or differences in the pathogenicity of the rust.

While the rust resistance of *Tutsan* complicates efforts to eradicate it as a weed, this characteristic also presents an opportunity. Given its status as a high-value commercial plant, *Tutsan*'s leaf rust disease resistance allows for the cultivation of healthy plants without the need for costly and environmentally harmful fungicides.

Berry and Leaf Characteristics

In the current study, Parent 2 exhibited rust-free leaves (Table 5), making it a promising candidate for rust resistance. However, this cultivar lacks other desirable ornamental traits, such as larger, deeper-colored berries and aesthetically appealing leaf characteristics. To address this limitation, crossbreeding is essential to combine the rustresistance traits from Parent 2 with the ornamental traits found in other cultivars. In this study, we examine two parental genotypes: the commercial cultivar, which has larger berries and deep green leaves but is susceptible to rust with light red berries, and the weedy genotype, which has smaller berries and light green leaves but exhibits resistance to rust and deep red berries, which are preferred for ornamental purposes. A cross between these two genotypes resulted in progeny displaying various combinations of these four traits (Table 1 and 5).

Among the progeny, Progeny One exhibited the ideal combination of traits: larger, deep red berries and deep green, rust-free leaves (Table 1 and 5). This combination enhances both the ornamental appeal and rust resistance, which is the desired outcome of the breeding program. We chose to vegetatively propagate this progeny through stem cuttings to preserve the desired traits without introducing genetic variability that could arise from sexual reproduction. Vegetative propagation ensures the offspring remain genetically identical to Progeny One, thus maintaining the combination of

deep red berries, larger berry size, deep green leaves, and rust resistance. Furthermore, this method avoids the risk of undesirable traits reappearing, which can happen when seeds are used for propagation (Trueblood et al., 2010; Beeresha et al., 2024).

Table 1. Genotypes of *Hypericum androsaemum* grown at the Milko Flower Farm experimental field in the Gurage Zone of Central Ethiopia during the year 2022 in two cycles January to May and June to September and evaluated for, berry color, berry size, and leaf color.

No	Genotypes	Berry color	Berry size	Leaf color
1	Progeny 1	Deep red	Large	Deep Green
2	Progeny 2	Deep red	Large	Deep Green
3	Progeny 3	Light red	Large	Deep Green
4	Progeny 4	Light red	Small	Deep Green
5	Progeny 5	Light red	Small	Deep Green
6	Commercial cultivar (Parent 1)	Light red	Large	Deep Green
7	Weedy genotype (Parent 2)	Deep red	Small	Light Green

Days to Flowering and Stem Length (cm)

The genotypes exhibited very highly significant differences in the number of days to flowering (P < 0.001, Table 2). This variation is expected because the genotypes are heterogeneous, meaning they consist of different genetic makeups. Both parent plants flowered significantly earlier than all progenies, except for Progeny 4 (Table 5). Progenies 1 and 2 exhibited significantly later flowering compared to all other genotypes. Additionally, highly significant differences in stem length were observed (P < 0.001, Table 3). Progenies 1 and 2 had significantly longer stems than the other genotypes, although the difference in stem length between these two progenies was not significant (Table 5). While earlier flowering is

generally preferred, it is often associated with shorter and less vigorous plants compared to those that flower later. Genotype 1, which flowers later and has a longer stem, appears to be a promising candidate for cultivation. Longer-stemmed plants tend to have higher market value, making Genotype 1 potentially more profitable than the earlierflowering, shorter-stemmed genotypes. When such heterogeneous genotypes are propagated by seed rather than through vegetative propagation, the offspring can exhibit a wider range of traits, including flowering time. This phenomenon is consistent with findings by Trueblood et al. (2010), which highlight how seed propagation can lead to greater variability in traits.

Source of variation	DF	SS	MS	P-Value
Genotypes	6	254.6	42.4	0.000
Block	2	2.0	1.0	0.397
Error	12	12.0	1.0	
Total	20	268.6		

Table	3. A	nalvsis	of V	ariance	for	stem	length
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Source of Variation	DF	SS	MS	P-Value
Genotypes	6	124.6	20.8	0.000
Bock	2	0.7	0.3	0.619
Error	12	8.0	0.7	
Total	20	133.2		

Tuble 4. That yos of variance for real rust meets ty (70)					
Source of Variation	DF	SS	MS	P-Value	
Genotypes	6	18271.6	3045.3	0.000	
Bock	2	103.2	51.6	0.271	
Error	12	424.1	35.34		
Total	20	18799.0			

 Table 4. Analysis of Variance for leaf rust intensity (%)

Table 5. Mean of days to flowering, stem length and leaf rust intensity (percent leaf coverage by rust) of the studied Genotypes of *H. androsaemum* grown at the Milko Flower Farm experimental field in the Gurage Zone of Central Ethiopia during the year 2022 in two cycles January to May and June to September

No	Genotypes	Days to flowering	Stem length	Rust intensity %
1	Progeny 1	59a	41.0a	91.7a
2	Progeny 2	60a	38.7ab	63.3b
3	Progeny 3	56c	38.0b	45.0c
4	Progeny 4	51d	35.3c	38.3c
5	Progeny 5	57bc	36.3bc	36.7c
6	Parent 1	51d	35.3c	2.7d
7	Parent 2	51d	34.3c	2.0d
	Mean	55	37	40
	LSD _{5%}	1.8	1.5	10.8

The mean followed by the same letter (s) in the same column are not significantly different at the 5% level of significance

CONCLUSIONS

The cross-pollination between the leaf rustsusceptible commercial cultivar and the resistant weedy genotype of Hypericum androsaemum resulted in the production of viable F1 seeds. The viability of these seeds, combined with the presence of weedy plants near flower farms where this species is not native, raises concerns about the potential for invasive growth. This underscores the need for measures to prevent the unintended spread of this cultivar. One of the F1 progenies exhibited several desirable ornamental traits, including rust resistance and deep red berries inherited from the weedy parent, as well as larger berries and deep green leaves from the commercial variety. Additionally, the progeny displayed longer stems than either parent. The progeny consistently exhibited distinct, uniform characteristics, making it suitable for commercial use as a new cultivar. This cultivar has been named H. androsaemum 'Ruby Excess', registered, and assigned a product code by Floricode. It is currently being cultivated on a commercial scale for export. The introduction of this rust-resistant cultivar offers the potential to reduce fungicide use, thus lowering associated costs and mitigating the environmental impact of fungicide application. Furthermore, the methodology used in this study provides a framework for introducing leaf rust resistance into other commercial varieties of *H. androsaemum.*

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