

Variation of morpho-agronomical characteristics and genetic diversity of imported asparagus cultivars in Vietnam

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Abstract

Asparagus (*Asparagus officinalis* L.) is a perennial plant globally known for its unique texture, good flavor, and high nutritional value. This paper aims to provide information on the phenotypic traits, the situation of stem blight disease of the imported asparagus varieties and introduce suitable asparagus cultivars for Vietnam. The evaluation of morpho-agronomical characteristics variability of 13 imported asparagus cultivars in the first growing year was arranged in a Random Complete Block Design (RCBD) with 3 replicates in Hanoi, Vietnam. Genetic diversity was assessed using hierarchical clustering on principal component analysis of all measured traits. The results showed that 13 asparagus cultivars were classified into 4 main groups matched with geographical origins based on principal component analysis. Atlas achieved the highest harvested yield (11.4 quintals ha⁻¹) in the first harvesting time, which is correlated with high root CHOs level, followed by UC157, WB231 and Grande varieties. Purple passion had the highest sugar and vitamin C content. However, all varieties were infected with stem blight disease. In general, Atlas could be recommended for asparagus cultivation in Vietnam with high yield and good quality.

Keywords: asparagus, diversity, yield of spears, stem blight disease, CHOs.

Abbreviation: CHO_carbohydrate, MAP_months after planting, PC_principal component, TOEOS_Time of emergence of spears, ACOA_Anthocyanin coloration of apex, SOA_Shape of apex, BOBCS_Diameter of base of apex compared to middle of stem, AOB_Attitude of bracts, OFB_Opening of bracts, DOP_Density of phylloclades, IOGCOF_Intensity of green coloration of foliage, TOBOF_Time of beginning of flowering, TOF_Type of flowering, LOFBBOA_Length of first bracts at base of apex, WOFBBOA_Width of first bracts at base of apex, NOS_Number of stems, LOS_Length of stem, LUTFR_Length up to first ramification, DGL_Diameter at ground level, SC_Color of Stem.

Introduction

Asparagus (*Asparagus officinalis* L.) is a perennial, economic important horticultural crop (Sarabi et al., 2010). Asparagus is well-known as the 'King of Vegetables' because of its unique texture, flavor and high nutritional content (Baxter et al., 2003). Asparagus is a rich source of protein, selenium, choline, ascorbic acid, vitamins B, saponin and other phenolic compounds, which are accountable for various bioactive characteristics (del Arbol et al., 2016; Guo et al., 2020).

Asparagus is grown widely over the world mainly in South and Central America, China and Europe (Prohens et al., 2008). Asparagus productivity varies greatly which depends on the climatic conditions of each region and production techniques (Drost, 2023). China has still become the biggest asparagus producer and exporter in the world with nearly 90,000ha in 2020 (Drost, 2023). The highest spear yield is in Peru and Mexico with 11,670 and 9,430 kg ha⁻¹, respectively. However, average yields of other regions only achieved half

of the yield of the highest production areas which are 5,344 kg ha⁻¹ in Asia, Europe (5,010 kg ha⁻¹), North America (4,146 kg ha⁻¹), and Australasia (4,247 kg ha⁻¹) (Drost, 2023). Asparagus is adaptable to temperate regions where low temperatures or drought give the crop a dormant period (Pham, 2018). The optimal temperature is 24°C-29°C day and 13°C-19°C night, which is favor high productivity and longevity (Drost, 2020). At temperatures lower or higher than the optimum, growth are slower or assimilation rates decrease significantly (Bai and Kelly, 1999; Yen et al., 1992). Therefore, the cultivars must be evaluated in the zone where they will be introduced (González, 2006).

Morphological characteristics and molecular markers is usually used for genetic diversity assessment (Muthusamy et al., 2008; Amato et al., 2021). Morphological characterization is the traditional method used for assessment of genetic resource diversity in economy, simple and intuitional way (Vithanage et al., 1995; Linda et al.,

2009). On the other side, the evaluation for agronomic traits, quality parameters and resistance to abiotic and biotic stresses is fundamental for the identification of good genetic resources for development (Tripathi et al., 2018). The screening of productivity varieties of asparagus commonly used important characteristics including high total yield, high-quality spears (tight tips; uniform shape), good spear thickness, and improved earliness (Van den Broeck and Boonen, 1990). Therefore, the evaluation of morphological variation related to genetic diversity and of agronomic traits of asparagus varieties in changing environments can provide valuable information for breeding programs (Moose and Mumm 2008).

In Vietnam, green asparagus is one of the popular imported vegetables because of its high nutritional content and valuable source of income for the producer (La et al., 2022). Due to the rapid development in recent years, asparagus growing area in Vietnam has increased rapidly, spreading across the country like Ninh Thuan province, Ho Chi Minh City, Lam Dong province, Hai Phong, Hai Duong,... In which, Ninh Thuan province is the largest asparagus growing area reaching 426 ha at the end of 2022. In addition, a number of other provinces in the South Central region such as Quang Nam, Quang Ngai, Binh Dinh, Khanh Hoa and Binh Thuan are tending to expand the area of asparagus growing, because asparagus is a suitable crop for soil and climatic conditions in the regions (Phuong and Hau, 2022). The Northern Vietnam where has great potential for the consumption of asparagus, however, is still limited because it lacks of research for evaluation, selection and identification of adaptable varieties. In Vietnam, most of the imported asparagus varieties mainly originate from the US and Netherlands where the climate is temperate (Pham, 2018), while the North of Vietnam is hot and humid condition and susceptible to stem blight disease (*Phomopsis asparagi*). In 2018, 70% of growing asparagus in the North suffered from stem blight disease (Mai et al., 2020). In our study, all imported varieties originated from the US and Netherlands therefore the purpose of study is to examine the performance of imported asparagus varieties in terms of growth, yield, *Phomopsis asparagi* disease tolerance and quality of asparagus spear in order to introduce varieties with characteristics suitable Vietnam as well as classify the diversity of asparagus genetic resources based on morphological characteristics to evaluate good genetic resource for production.

Results and Discussion

Morpho-agronomical characteristics of imported asparagus varieties

The time of emergence of spears in Purple passion variety was later than other cultivars. Many cultivars had anthocyanin coloration of apexe like Atlas, WB231, UC115, Purple passion, K767, WB233, WB230 and WB208. The shape of apex ranged from narrow to broad triangular (Table 1). The length and width of the first bracts at base of apex ranged 7.5-11.3 and 6.12-13mm, respectively. The number of stems at 9 months after planting of the varieties in the group ranged from 6.3-13.7 stems/clusters. The length of stem was highest in WB231 with 203.3cm and lowest in Netherland cultivars (Vegalim, Lunalim and K767). The length up to the first ramification of cultivars ranged from 45.4 to 70.2cm. Atlas and UC115 had the biggest diameter at ground level, and the lowest in Vegalim (Table 2). Only the Purple passion variety had a purple stem, the rest of the

varieties were green. Three Netherland cultivars have bisexual flowers but do not produce fruits, which were announced as male flowers. The flower type of WB208 was male. The remaining varieties were plants with bisexual flowers type including both ovary and ovules which can produce fruits (Table 1).

Physiological characteristics of asparagus

Brix of root was highest in Atlas cultivar, but there were no significant differences between UC157, Grande, WB230, WB231, Vegalim, K767 and Purple passion cultivar. The variety with the lowest Brix was WB233 with 13.5%, no significant difference from Lunalim with 14.2%. Root carbohydrate (CHOs) results were similar trends to Brix root content (Table 3).

The content of Chlorophyll a did not differ between cultivars, while chlorophyll b reached the highest in Purple passion cultivar and the lowest in WB231. Chlorophyll a+b gives the same results as Chlorophyll b (Table 3). These data explained the similar green color of all imported cultivars in the field, except the Purple passion cultivar had a slightly darkened green.

Disease incidence of stem blight

All tested cultivars were susceptible to *Phomopsis asparagi*, which causes stem blight, a disease that causes asparagus stems and branches dried in the sub-tropic and tropic regions. We observed the disease severity at 3, 6 and 9 months after planting (MAP). The 6 and 9MAP records were conducted after two months with hot temperatures and the highest rainfall and storms (May and August). The result showed that at 3MAP almost cultivars were not infected Stem blight disease (*Phormosis asparagi*). Three cultivars had disease rate <10% including Grande, Vegalim, and UC115. Two cultivars with a disease rate of over 10% were WB208 and Lunarlim. At 6MAP, after rainstorms and high temperatures, the rate of disease increased significantly. The disease rate was recorded highest in Grande (34.8%) and around 30% in almost other varieties. Only Purple passion variety had a disease rate of less than 20%. At 9MAP, the disease rate increased again, almost the cultivars were infected severely with the disease incidence reaching around 50%. Two cultivars had the lowest disease incidence were Purple passion and Atlas with 40.7 and 39.0%, respectively (Figure 1).

Yield and yield components of asparagus cultivars

The results of yield and yield components of asparagus cultivars grown were presented in Table 4. The diameter of spears in the first year harvest was highest in Atals, followed by Grande and the lowest were Vegalim and Lunalim. The length of spear at the first harvesting time did not differ significantly between the imported cultivars.

The number of spears/plant/month of harvested cultivars ranged from 4.3-8.0 spear/plant, the highest in the Atlas cultivar and the lowest in Purple passion, the other cultivars did not detect any differences in the number of spear/plant. The average spear weight was highest in the Atlas cultivar, followed by Grande, UC157, WB231. The lowest spear weight was found in the cultivars Vegalim and WB208.

As a consequence, the individual yield of the varieties ranged from 39.3-83.1g, the highest in the Atlas cultivar and the lowest in the Purple Passion cultivar. The first time harvested yield was highest in Atlas with 11.4 quintal ha⁻¹, followed by WB231 and UC157 (about 10.0 quintal ha⁻¹). The

lowest yields were WB208 and Purple Passion (7.5 quintal ha⁻¹) and Vegalim (6.7 quintal ha⁻¹).

Quality of asparagus spears

The quality of asparagus spears of each cultivar was presented in Table 5. The dry matter of the cultivars ranged from 6.5-8.2g/100g fresh weight, of which the highest was Atlas however it was similar statistically with WB212, UC157, UC115, WB230, K767, Purple passion. Brix spear ranged from 5.3-6.2%, the two cultivars with the highest Brix were UC157 and WB230 (about 6.2%), followed by WB233 and Purple Passion (6%).

Vitamin C content reached the highest in Purple Passion cultivar with 18.9mg, the lowest in Grande and WB208 reached 7.7 and 7.8mg, respectively. The total sugar content did not differ among cultivars in which Purple Passion was the highest value with 3.3%. The lowest fiber content recorded in Atlas (1.54%), the highest one was WB233 cultivar reaching 2.33% and the remaining cultivars fluctuated among 1.76-2.3%.

Spears' size at harvest time is an important factor in marketing. The results in Figure 2 showed that at the first time harvest, there were no varieties that had grade 1 spear. The percentage of grade 2 spear was also low ranging from 0-8.5%, the highest one in Purple passion with 8.5%. Most of the cultivars harvested with grade 3 spears, accounting for around 80%, the highest rate was Atlas with 86.5%, followed by UC157 with 83.5% (Figure 2). The grade 4 included spears with diameters from 4 - 6.9 mm (about 9.0 – 20.1% among cultivars), which could sell at low price in the market. The lowest rate was recorded in Atlas and the highest in WB212, WB208 and Lunelim.

Morphological diversity of imported asparagus varieties

Phenotypic assessment is an important method for selecting and classifying genetic resources and plant varieties. In this study, principal component analysis was applied to reduce the dimensions of agronomic traits into principal components (PCs). The sum of the first two major components contributed 62.5% of the total phenotypic variation (Figure 3). Traits that contribute largely to the main components of the PC1 and PC2 axis were considered useful traits for asparagus gene pooling. Thus, five traits including SC (Color of Stem), IOGCOF (Intensity of green coloration of foliage), TOEOS (Time of emergence of spears), NOS (Number of stems), LOS (Length of stem) contributed >8% to the PC1 axis (Figure 3) and five traits including TOF (Type of flowering), LUTFR (Length up to first ramification), WOFBBOA (Width of first bracts at base of apex), DGL (Diameter at ground level) and LOS (Length of stem) contributed >8% to PC2 axis (Figure 3).

Hierarchical clustering based on principal components allows a combination of three kinds of methods used in multivariate data analysis including principal components, hierarchical clustering, and partitional clustering using the k-means algorithm allows to better highlight and better describe the resemblances between individuals (Husson et al., 2010). The hierarchical clustering on principal components divided 13 asparagus cultivars into 4 main groups: the first group (Group 1) was separated from all the others and contained only Purple passion cultivar; the second group cluster (Group 2 including Vegalim, Lunelim and K767 cultivar) was formed by cultivars of Netherland origin; Group 3 and Group 4 were of USA origin, in which Group 3 including UC115, WB231, Atlas, Grande, UC157,

WB212 and Group 4 including WB208, WB230, WB233 (Figure 4).

Table 6 showed the average values of cultivars in four clustering groups for 9 morphological traits that had a great contribution to cluster (Figure 4). The data presented clearly differences in morphological traits among groups. Group 3 and Group 4 included the varieties with high yield of which the number of spears (NOS) and the length up to first ramification (LUTFR) were high and the length of spears (LOS) was tall.

Discussion

Evaluation of genetic resource diversity provides information for plant breeders to develop new and improved cultivars with desirable characteristics (Govindaraj et al., 2015). In our study, 9 morphological traits including stem color (SC), length of stem (LOS), width of first bracts at base of apex (WOFBBOA), length up to first ramification (LUTFR), intensity of green coloration of foliage (IOGCOF), spear diameter at ground level (DGL) and number of stems (NOS) explained 62.5% of the total phenotypic variation (Figure 5). Four traits (width of first bracts at base of apex (WOFBBOA), length up to first ramification (LUTFR), spear diameter at ground level (DGL) and the number of stems (NOS) were used among 9 morphological characteristics that explained 83.37% of the total variance using the principal in clustering 46 accessions in the study of Chen et al., (2020). Our study pointed out that the varieties (Group 3 and Group 4) had high yield which had characteristics of a high number of spear (NOS), length up to first ramification (LUTFR) and length of spear (LOS) (Table 6). The result was consistent with the findings of Chen et al., (2020) who said that for cultivation of a high-yield variety of asparagus, length up to first ramification, diameter of main stem, spear diameter, spears weight and diameter of primary branches, dense of phylloclades should be taken as the primary indexes. The results of our clustering genetic distance based on principal components showed that 13 varieties of asparagus were classified into 4 main groups (Figure 4), matched with geographical origin of which Group 2 contained the cultivars from Netherlands and Group 3 and Group 4 included cultivars from the US. This result was consistent with Caruso et al., (2007) who used eight EST-SSR loci differentiated into various clusters and found that the seven *Asparagus* plant species closely corresponded to the geographical origin. This was also agreed with Chen et al., (2020) found that the cultivars came from of the same origin mostly form a cluster together and they may be derived from common ancestors. The study shows that the morphological characteristics are useful to classify of genetic diversity of asparagus and can choose the promising high-yield varieties based on morphological characteristics which can be used in breeding programs.

The physiology of yield determination in asparagus is complex because it is a perennial crop. The level of root-stored carbohydrates (CHOs) is one key indicator of yield potential (Wilson et al., 2002; Paschold et al., 2008; Reomero-Vergel, 2023). Asparagus accumulated water-soluble carbohydrates (WSC) mainly in the freshly adventitious roots which are exported from the canopy cell through the phloem to the root, after that WSC can be used in spear and stem development (Shiomi, 1992). Researchers and growers have been using Brix degree in root to monitor WSC content to quantify asparagus productivity (Wilson,

Table 1. Morphological characteristics of asparagus cultivars.

Characteristics	ACOA	SOA	DOBCS	AOB	OFB	DOP	IOGCOFF	TOF	SC
Atlas	9	1	3	2	3	5	5	4	1
Grande	1	2	3	2	3	5	5	4	1
WB231	9	1	3	2	3	5	5	4	1
WB212	1	1	3	2	3	5	5	4	1
UC157	1	1	3	2	3	5	5	4	1
UC115	9	1	3	2	3	5	5	4	1
Purple passion	9	1	3	2	3	5	7	4	9
Vegalim	1	2	3	2	3	5	5	5	1
Lunalim	1	1	3	2	3	5	5	5	1
K767	9	1	3	2	3	5	5	5	1
WB233	9	2	3	2	3	5	5	4	1
WB230	9	3	3	2	3	5	5	4	1
WB208	9	3	3	2	3	5	5	6	1

Notes: Anthocyanin coloration of apex (ACOA), Shape of apex (SOA), Diameter of base of apex compared to middle of stem (DOBCS), Attitude of bracts (AOB), Opening of bracts (OFB), Density of phylloclades (DOP), Intensity of green coloration of foliage (IOGCOFF), Type of flowering (TOF), Color of Stem (SC).

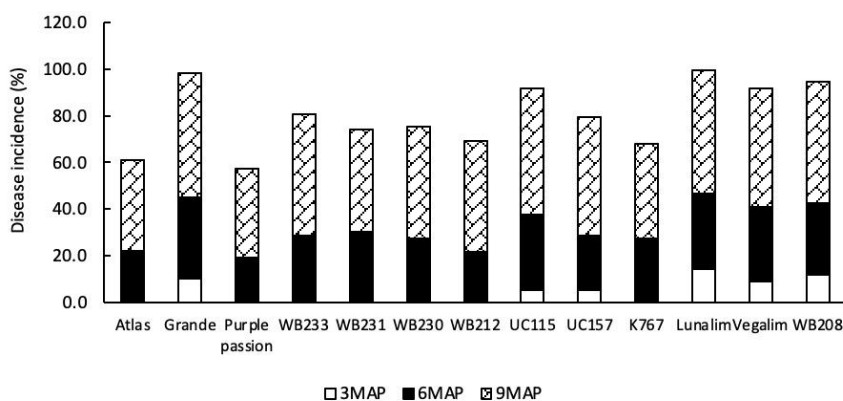


Figure 1. Disease incidence of stem blight of asparagus cultivars. Note: MAP_months after planting.

Table 2. Agronomical characteristics of asparagus cultivars.

Characteristics	TOEOS	TOBOF	LOFBBOA	WOFBBOA	NOS	LOS	LUTFR	DGL
Atlas	5	5	9.32	9.13	10.6	190.4	66.9	8.3
Grande	5	5	10.09	7.17	11.7	185.1	64.8	7.9
WB231	5	5	9.94	8.16	10.4	203.3	70.2	7.8
WB212	5	5	9.93	9.04	9.8	184.8	67.3	7.6
UC157	5	5	9.98	7.52	11.9	178.6	61.6	7.8
UC115	5	5	8.47	7.98	10.9	184.8	66.1	8.3
Purple passion	7	5	10.00	9.08	6.3	153.5	53.3	8.0
Vegalim	5	5	11.30	13.00	9.1	159.8	47.8	7.1
Lunalim	5	5	7.5	10	10.8	154.7	49.0	7.6
K767	5	5	9.40	12.70	8.4	156.4	52.0	7.7
WB233	5	5	8.18	6.38	12.4	188.8	58.3	7.2
WB230	5	5	9.22	6.12	11.9	174.9	62.0	7.4
WB208	5	5	9.20	11.00	13.7	174.6	45.4	7.7

Notes: Time of emergence of spears (TOEOS), Time of beginning of flowering (TOBOF), Length of first bracts at base of apex (LOFBBOA), Width of first bracts at base of apex (WOFBBOA), Number of stems (NOS), Length of stem (LOS), Length up to first ramification (LUTFR), Diameter at ground level (DGL).

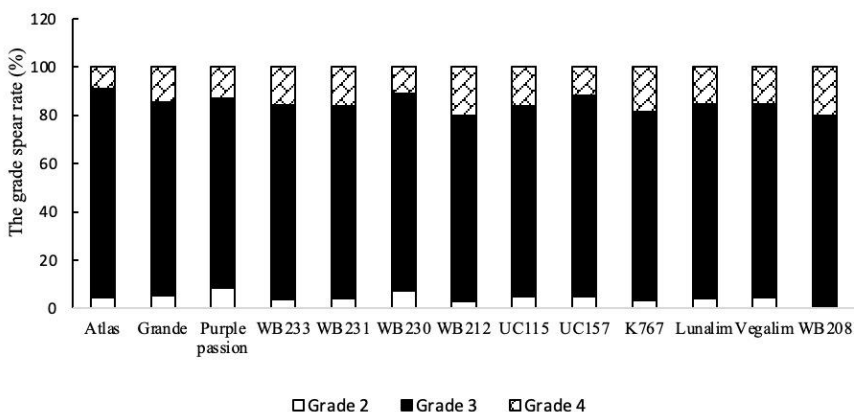


Figure 2. The percentage of harvested spear grades of asparagus cultivars.

Table 3. Some physiological characteristics of asparagus cultivars.

Cultivar	Root brix (%)	Carbohydrate (mg/kg)	Chlorophyll a	Chlorophyll b	Chlorophyll a + b
Atlas	16.5 ^a	391.1 ^a	30.3 ^a	66.5 ^a	96.5 ^a
WB212	14.1 ^{bc}	340.4 ^{bc}	30.4 ^a	64.0 ^{ab}	94.1 ^{ab}
UC157	15.8 ^a	377.0 ^a	31.3 ^a	59.9 ^{ab}	90.9 ^{ab}
UC115	15.2 ^{ab}	364.3 ^{ab}	30.2 ^a	62.4 ^{ab}	92.2 ^{ab}
Grande	16.4 ^a	382.6 ^a	30.9 ^a	62.1 ^{ab}	92.6 ^{ab}
WB230	15.4 ^{ab}	367.8 ^{ab}	31.4 ^a	61.5 ^{ab}	92.6 ^{ab}
WB208	13.5 ^c	327.0 ^c	29.6 ^a	67.9 ^a	97.1 ^a
WB231	15.9 ^a	378.4 ^a	32.2 ^a	53.8 ^b	85.7 ^b
WB233	13.5 ^c	328.5 ^c	31.5 ^a	61.4 ^{ab}	92.6 ^{ab}
Vegalim	15.2 ^{ab}	363.6 ^{ab}	31.4 ^a	61.4 ^{ab}	92.5 ^{ab}
K767	15.9 ^a	379.1 ^a	30.5 ^a	65.1 ^{ab}	95.2 ^a
Lunalim	14.2 ^{bc}	343.2 ^{bc}	31.2 ^a	62.0 ^{ab}	92.9 ^{ab}
Purple passion	15.2 ^{ab}	364.3 ^{ab}	30.0 ^a	68.2 ^a	97.8 ^a

Mean followed by different letters within a column are significantly different at 0.05 probability and vice-versa.

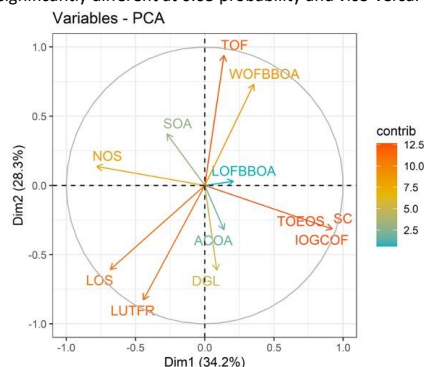


Figure 3. Principal Components Analysis (PCA) of monitored traits of asparagus cultivars.

Table 4. Yield and yield components of asparagus cultivars.

Cultivar	Length spear (cm)	Diameter spear (mm)	Number spear/plant	of Spear weight (g)	Individual yield (g/plant)	Harvested yield (quintal/ha)
Atlas	25.3 ^b	8.32 ^a	8.0 ^a	10.4 ^a	83.1 ^a	11.4 ^a
WB212	26.2 ^{ab}	7.53 ^{abcd}	6.6 ^b	9.5 ^{bcd}	61.8 ^{bc}	9.6 ^{bc}
UC157	29.0 ^{ab}	7.97 ^{abc}	6.4 ^b	9.9 ^{abc}	63.9 ^b	10.0 ^b
UC115	25.5 ^{ab}	7.13 ^{def}	6.2 ^b	9.3 ^{cde}	57.8 ^{bc}	9.4 ^{bc}
Grande	26.2 ^{ab}	8.15 ^{ab}	6.2 ^b	10.0 ^{ab}	62.3 ^{bc}	9.9 ^{bc}
WB230	26.6 ^{ab}	7.18 ^{de}	6.2 ^b	9.5 ^{bcd}	59.0 ^{bc}	9.5 ^{bc}
WB208	26.0 ^{ab}	7.43 ^{cde}	5.9 ^b	7.9 ^{fg}	47.1 ^{de}	7.5 ^{ef}
WB231	27.2 ^{ab}	8.0 ^{abc}	6.3 ^b	9.8 ^{abcd}	61.3 ^{bc}	10.1 ^b
WB233	27.0 ^{ab}	7.43 ^{bcd}	5.8 ^b	9.2 ^{cde}	54.0 ^{bcd}	9.0 ^{cd}
Vegalim	27.5 ^{ab}	6.40 ^f	5.9 ^b	7.5 ^g	44.7 ^{de}	6.7 ^f
K767	27.5 ^{ab}	7.97 ^{abc}	6.6 ^b	9.6 ^{bcd}	63.3 ^b	9.9 ^{bc}
Lunalim	29.8 ^a	6.63 ^{ef}	6.1 ^b	8.7 ^{ef}	52.9 ^{cd}	8.0 ^{de}
Purple passion	25.0 ^b	7.60 ^{abcd}	4.3 ^c	9.1 ^{de}	39.3 ^e	7.5 ^{ef}

Mean followed by different letters within a column are significantly different at 0.05 probability and vice-versa.

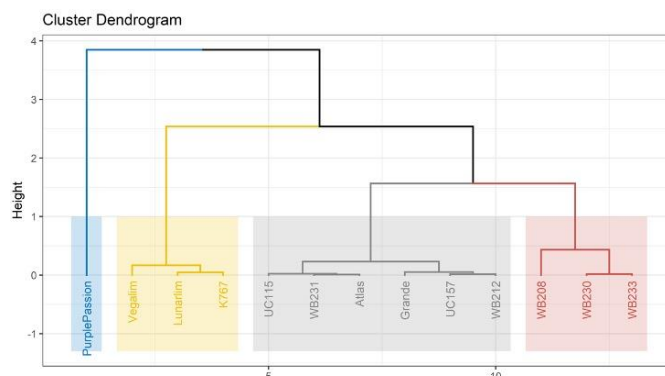


Figure 4. Principal component-based clustering of asparagus cultivars.

Table 5. Quality characteristics of spears of asparagus cultivars.

Cultivar	Dry matter (g/100g fresh)	Spear brix (%)	Vitamin C(mg)	Total sugar (%)	Fiber content (%)
Atlas	8.2 ^a	5.6 ^{bcd}	13.3 ^{cd}	2.4 ^b	1.54 ^k
WB212	7.9 ^{abc}	5.6 ^{bcd}	11.8 ^{de}	2.3 ^{bc}	1.89 ^h
UC157	7.7 ^{abc}	6.2 ^a	13.3 ^{cd}	2.0 ^d	2.30 ^b
UC115	7.8 ^{abc}	5.6 ^{cd}	15.8 ^b	2.2 ^c	1.89 ^h
Grande	7.3 ^{abc}	5.5 ^d	7.7 ^h	2.3 ^{bc}	2.06 ^e
WB230	8.2 ^a	6.2 ^a	13.7 ^c	2.4 ^b	2.25 ^c
WB208	7.5 ^{abc}	5.3 ^d	7.8 ^h	2.2 ^c	1.76 ^j
WB231	7.4 ^{abc}	5.5 ^d	8.5 ^{gh}	2.0 ^d	1.97 ^g
WB233	6.5 ^c	6.0 ^{abc}	13.3 ^{cd}	2.1 ^d	2.33 ^a
Vegalim	6.5 ^c	5.6 ^{cd}	11.3 ^{ef}	1.8 ^e	1.85 ⁱ
K767	8.1 ^a	5.5 ^d	8.6 ^{gh}	2.0 ^d	2.09 ^d
Lunalim	6.6 ^{bc}	5.6 ^{bcd}	10.0 ^{fg}	1.5 ^f	2.0 ^f
Purple passion	8.0 ^{ab}	6.0 ^{ab}	18.9 ^a	3.3 ^a	2.11 ^d

Mean followed by different letters within a column are significantly different at 0.05 probability and vice-versa

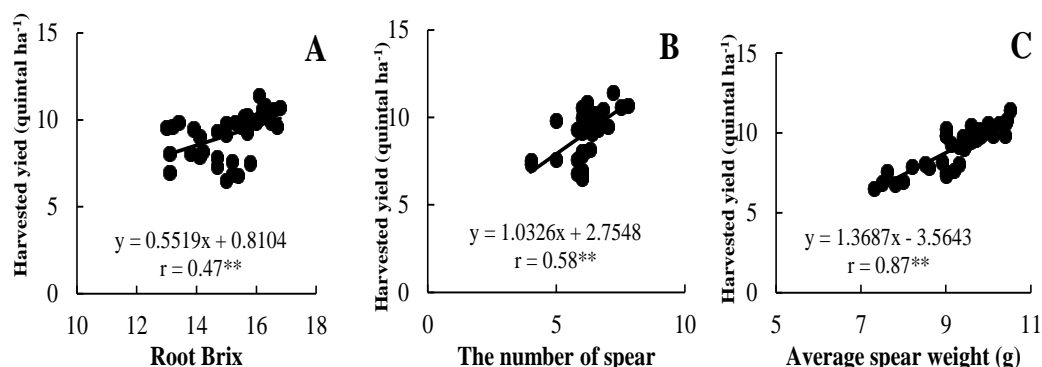


Figure 5. Relationships between root Brix (A), the number of spears (B) and average weight of spear (C) with harvested yield of Asparagus cultivars. **** significant difference at $P \leq 0.01$, respectively.**

Table 6. Mean value of nine morphological traits of 4 clustering groups.

Characteristics	Group 1	Group 2	Group 3	Group 4
SC	9	1	1	1
IOGCOF	7	5	5	5
TOEOS	7	5	5	5
NOS	6.3	9.4	10.9	11.7
LOS (cm)	153.5	157.0	187.8	179.4
TOF	5	4	5	5
LUTFR (cm)	55.3	49.6	66.1	52.2
WOFBBOA (mm)	9.1	11.9	8.2	7.8
DGL (mm)	8.0	7.4	8.0	7.4

Notes: Color of Stem (SC): 1 green, 9 purple; Intensity of green coloration of foliage (IOGCOF): 3 light, 5 medium, 7 dark; Time of emergence of spears (TOEOS): 3 early, 5 medium, 7 late; Number of stems (NOS); Type of flowering (TOF): 4: androhermaphrodite flower+ovary+ovule; 5: androhermaphrodite flower+ovary+without ovule; Length up to first ramification (LUTFR); Width of first bracts at base of apex (WOFBBOA); Diameter at ground level (DGL).

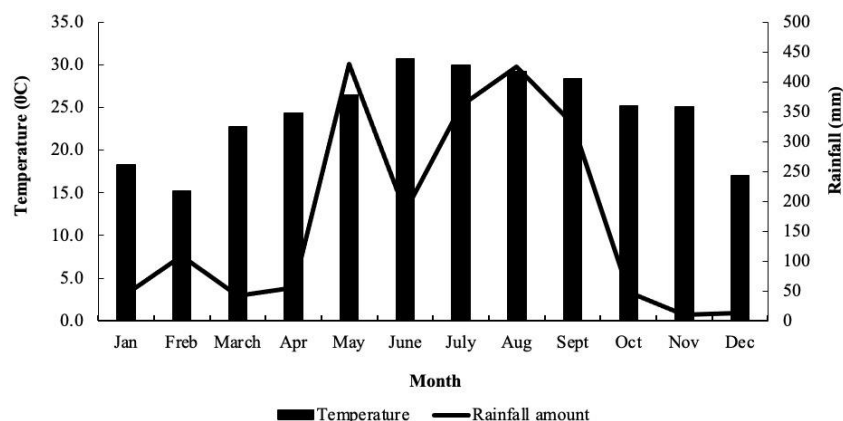


Figure 6. Temperature and rainfall during the experimental period. (Source: Hanoi hydrometeorological station, 2022).

Table 7. Some soil chemical characteristics of the experimental field.

No.	Parameters	Unit	Value	No.	Parameters	Unit	Value
1	pH		7.86	6	Available N	mg/100g	4.5
2	Organic carbon (OC)	%	1.15	7	Available P ₂ O ₅	mg/100g	13.1
3	Total N	%	0.15	8	Available K ₂ O	mg/100g	14.4
4	Total P ₂ O ₅	%	0.16	9	Available Ca ²⁺	mg/100g	397.7
5	Total K ₂ O	%	2.20	10	Available Mg ²⁺	mg/100g	25.2
				11	Available Fe ²⁺	mg/100g	15.7

(Source: VNUA soil and fertilizer analytical laboratory, 2022).

Table 8. Monitoring morphological characteristics of asparagus.

No.	Characteristics	Abbreviate	Measurement/Status	Score
1	Density of phylloclades	FOP	Sparse/Medium/Dense	3/5/7
2	Intensity of green coloration of foliage	IOGCOF	Light/Medium/Dark	3/5/7
3	Number of stems	NOS	Measure 10 plants per each replicate	
4	Length of stem	LOS	Measure 10 plants per each replicate	
5	Length up to first ramification	LUTFR	Measure 10 plants per each replicate	
6	Diameter at ground level	DGL	Measure 10 plants per each replicate	
7	Time of emergence of spears	TOEOS	Early/Medium/Late	3/5/7
8	Time of beginning of flowering	TOBOF	Early/Medium/Late	3/5/7
9	Type of flowering	TOF	Plant with male and female flower Plant with male flower and female flower Plant with androhermaphrodite flower Plant with androhermaphrodite flower with ovule and ovary Plant with androhermaphrodite flower with ovule and without ovary Male flower	1 2 3 4 5 6
10	Anthocyanin coloration of apex	ACOA	Absent/Present	1/9
11	Shape of apex	SOA	Narrow trianggular Medium trianggular Broad trianggular	1 2 3
12	Diameter of base of apex compared to middle of stem	DOBOACTMOS	Smaller/Equal/larger	1/2/3
13	Attitude of bracts	AOB	Adpressed Slightly held out Marketly held out	1 2 3
14	Length of first bracts at base of apex	LOFBBOA	Measure 10 spear per each replicate	
15	Width of first bracts at base of apex	WOFBBOA	Measure 10 spear per each replicate	
16	Opening of bracts	OFB	Weakly open Moderately open Strongly open	3 5 5

Source: UPOV, 2010.

2005; Romero-Vergel, 2023). In this study, we measured Brix roots to determine CHOs in asparagus roots, the results showed that the Atlas varieties had the highest value in CHOs of 391.1mg/kg, leading to a high number of spears and average weight of spear. As a consequence, it was also the variety with the highest harvested yield reaching 11,4 quintal ha⁻¹. The high Brix degree in root led to high yield of Atlas in our study. However, due to the infestations by *Phormosis* disease in the summer so the stem and fern of asparagus in our experiment were severely damaged which might limit the leaf photosynthesis and then the accumulations of CHOs in the root which resulted in a relatively low yield of all varieties. The correlations also found a significant and positive relationships between the Brix root with harvested yield, number of spears and average weight of spear with harvested yield with $r = 0,47^{**}$; $r = 0,59^{**}$; $r = 0,87^{**}$, respectively (Figure 5). This result is also consistent with the correlations in the studies of Wilson (2005), Drost (2023) and Romero-Vergel (2023). The selection of asparagus cultivars is important, it should be productive for several years and relatively tolerant to diseases (González, 2006; Benson, 1996). The cultivars must be evaluated in the zone where they will be introduced

(González, 2006). Three asparagus cultivars Atlas, Grande, Purple passion were created adapted to central California. The average yield in 8 years-trial in various locations in the US, the yields of Atlas and Grande from California showed 25-50% higher than UC157 cultivar which was one of the most planted green asparagus cultivar in the warm and temperate zones of the world, with Atlas (5.9 tons/ha) and Grande (6.9 tons/ha). While Purple passion reached the maximum of 4.35 tons/ha across 5 locations (Benson, 1996). Additionally, Atlas and Grande were resistant to several major diseases *Fusarium* and *Pucciana*, while Purple passion was susceptible to *Pucciana* (González, 2006; Benson, 1996). Similar results of Nurlaeny et al., (2019) and Drost (2023) shown that Atlas adapted to the tropical medium climate and American soil millvila silt loam with a yield of spear 2.1 ton/ha. The introduced imported varieties in our study are described as heat tolerant, suitable for tropical areas, have green leaves, tightly closed heads, good yield and quality. In Vietnam, Atlas cultivar growing in Ninh Thuan province (considered as the most favorable area for the growth and development of asparagus in Vietnam) had a high yield (with the first year harvesting yield reaching 3.57 tones/ha) and high percentage of grade 1,2 spears and good resistance to

some major pests and diseases (Nguyen et al., 2020). In the Red River Delta, K767 performed well in the yield (reaching 2.34 tones/ha in the first year of harvesting) with high grade 1 spear. However, it was susceptible to *Phomopsis asparagi* causing stem blight (Pham, 2018), a dangerous disease of Asparagus in Vietnam as well as many Asian and Pacific countries that growing Asparagus, such as Japan, China, Myanmar, Australia, New Zealand (Cheah et al., 2006; Zaw et al., 2017). In our study, all cultivars were severely infected with *Phormosis asparagi* due to several rainstorms and high temperatures in summer (Figure 6). Therefore, the yield of all cultivars was not high. However, Atlas still achieved the highest yield with 11.4 quintal/ha, followed by WB231, UC157 and K767 (Table 4). Purple passion cultivar had a low yield due to a low number of spear/plant (Table 4). Purple passion has been approved that it contained the novel anthocyanin with high antioxidant activities (Sakaguchi et al., 2008). In addition, the high rutin content, high chl a and b, and total carotenoid content were found in Purple passion variety (Sergio et al., 2021). In this study, Purple passion had higher quality characteristics (Vitamin C, total sugar and fiber content) (Table 5) than others, therefore, it could be introduced for different marketing purposes. In general, Atlas would be the promising variety in Vietnam that should be introduced for production areas as well as for breeding programs.

Materials and methods

Plant materials

The experiment assessed the morphological and agronomical traits of 13 F1 hybrids varieties of asparagus imported from the US and the Netherlands. The 10 American asparagus cultivars were Atlas, Grande, UC157, UC115, WB 233, WB230, WB231, WB212, WB208 and pupurple passion. The 3 Netherlands varieties were Vegalim, K767 and Lunalim.

Experimental design

The experiment was conducted at the Upland Field of the Faculty of Agronomy, Vietnam National University of Agriculture - VNUA (36°32' South latitude; 71°55' West), located in Hanoi, Vietnam (the typical soil and climatic conditions of Red River Delta area). The soil was sampled before planting and soil chemical properties were analyzed by TCVN (Vietnamese national standards) at VNUA soil and fertilizer analytical laboratory with characteristics as in Table 7. The temperature and rainfall data was collected for 12 months in 2022 from Hanoi Hydro-Meteoroloical was showed in Figure 6. The experiment was designed in Randomized Complete Block Design (RCBD) with 13 treatments corresponding to 13 cultivars of Asparagus, with 3 replicates, a total of 39 experimental plots. The area of each plot was 20m². The planting distance was 0.75m between rows and 0.4m between plants in the row, and a planting depth of 35cm, 2 rows/bed. The soil was fertilized with 30 tons of chicken compost + 150:150:150kg NPK + 30kg MgO/ha. The basal fertilization before planting with 100% compost +100% MgO + 50% of NPK and the rest were applied at 1, 3, and 6 months after planting. Asparagus was planted on 16th December, 2021 using 3 month seedlings after sowing seeds in pots in the greenhouse.

Measurements

- *Morphological characteristics* were measured at 9 months after transplanting (MAP). The morphological parameters of

the spear were assessed at the first harvesting time (10 MAP) when the spear reached a length of 27cm. The monitoring indicators and measurement methods are presented in Table 8 following the International union for the protection of new varieties of plants (UPOV) for Asparagus.- *Physiological characteristics*:

+ Chlorophyll a, b and a+b content in phylloclades at 9 MAP were measured according to the method of Wintermans and De Mots (1965). The chlorophyll a and b contents were analyzed by the adsorption spectral analysis method following leaf extractions in a 96% ethanol solution. One gram of matured fern leaf tissue (fresh weight at 30 days after spear establishment) was added to a closed falcon tube containing 10mL of ethanol and put in dark conditions within 48 hours to complete the extraction of the chlorophyll solution in the leaves. After that, the solution was measured using a UV-2900 spectrophotometer (Hitachi, Japan). Absorbance (OD) was measured at the wavelengths 665 and 649nm. The chlorophyll a and b and a+b contents were calculated according to the following equations:

Chlorophyll a = $13.7 \times A_{665} - 5.67 \times A_{649}$ ($\mu\text{g mL}^{-1}$),

Chlorophyll b = $25.8 \times A_{649} - 7.60 \times A_{665}$ ($\mu\text{g mL}^{-1}$), and

Chlorophyll a+b = $6.1 \times A_{665} + 20.04 \times A_{649}$ ($\mu\text{g mL}^{-1}$).

+ Carbohydrate content (CHO) in roots at 9 MAP through measuring Brix roots by Wilson et al., (2005) method according to the formular: CHO = $21.1 \times \text{Brix}\% + 42.9$ (mg g^{-1})
- *Disease status of Stem Blight by Phomoris asparagi*: assess the natural infection situation in the open field by counting the number of infected stems on random 10 cluster per 1 plot every two weeks, then calculate the average disease incidence by formula: disease rate = number of infected stems in cluster/ total number of stems in cluster

- *The yield and yield components*: at 10 MAP, when the mother plant is yellow, cut the entire stem of the mother plant and harvested emerging spears when the spear reached around 27cm length. Count the number of spears obtained/10 plants/plot and weigh all the spear of cluster to determine individual yield. Weigh all spears/plot during the harvesting time (30 days) to get harvested yield.

- *Quality characteristics*: Measured spear quality including insoluble dietary fiber (AOAC, 1996), Brix content by Milwaukee MA882 machine, total sugar content (Deshavath, 2020), and vitamin C (Nielsen, 2017).

+ Spears were classified based on their thickness. The thickness was reflected by the diameters of spear bases, which were measured after 1 week from the first intensive harvest for all the varieties. Spears were classified into three grades:

- Grade 1: diameters of spear bases ≥ 12.0 mm
- Grade 2: diameters of spear bases 10.0-11.9 mm
- Grade 3: diameters of spear bases 7.0 -9.9 mm
- Grade 4: diameters of spear bases 4.0 -6.9 mm

Data analysis

Principal component analysis and hierarchical clustering on principal components using packages "FactoMineR" and "factoextra" on R software 4.1.3. Analysis of variance (ANOVA) of agromical characteristics was performed using 'Minitab 16', the significance of mean values was analyzed using Tukey's test (0.05).

Conclusions

The study shows that the morphological characteristics are useful to classify of genetic diversity of asparagus. The

asparagus cultivars were classified into 4 main groups with geographical origins. All cultivars had relatively low yields in the first harvesting due to the stem blight disease problem. Among these, the Atlas cultivar had the highest harvested yield due to high root CHOs level which resulted in the highest number of spears and spear weight, followed by varieties UC157, WB231 and Grande. Purple passion had the lowest yield but the highest in sugar and vitamin C content, which can be introduced for commercial purposes. Further studies need to be done on the management of physiological characteristics related spear yield and of stem blight disease for asparagus production in the coming years.

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