

Characterization of a novel pistillody mutant in common wheat

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Abstract

Pistillody is the homeotic transformation of stamens into pistils or pistil-like structures in wheat. During the development of common wheat near-isogenic line (variety Chinese Spring) carrying *Pis1* gene, a pistillody plant was serendipitously observed at field. It was further self-pollinated several generations into a pure pistillody line HTS-1. Pistillody is of breeding significance as it results in more pistils in a floret and even more seed-setting. It can be seen with the naked eye, in which one to three stamens in florets of HTS-1 plants always turn into pistils or pistil-like structures. Light microscopy indicated that some completely transformed stamens show well-developed ovules with normal fertility. Scanning of young spikes with electron microscopy revealed that the homeotic transformation of stamens into pistil-like structures occurred during early-stage stamen development. Unlike those reported before, each plant of HTS-1 showed pistillody trait under normal cultivation conditions. But pistillody degrees varied among the spikelets at different positions of the spike and among the florets of different spikelets. Inheritance analysis of the pistillody trait was carried out by crossing HTS-1 with normal commercial wheat varieties: Mianmai 45, Chuanmai 44, Chuanmai 50, and Chuanmai 28. The F_2 and BC_1 population segregation revealed that the pistillody trait is determined by the interaction of two recessive karyogenes *hts1* and *hts2*. The special genetic base means that this HTS-1 is different from the previously reported lines (cr)-CSdt7BS and N26.

Keywords: Wheat, pistillody, pistil, stamen, homeotic transformation.

Abbreviations: TP_three-pistil; CSTP_Chinese Spring TP; cTs_completely transformed stamen; pTs_partially transformed stamen.

Introduction

The inflorescence of a wheat plant is called a spike, which located at the head of the stem. Wheat spike is composed of many spikelets arranged to form two opposite rows along the main axis (Shitsukawa et al., 2009). The spikelet is composed of several florets joined at the axis alternately on opposite sides. The floret can be considered as the flower of wheat because of its structure and function. An individual wheat floret contains one pistil, three stamens, and two lodicules. The pistil is the female part of the flower and consists of an ovary containing ovules and two filamentous styles, each terminating in a feathery stigma. The stamen is the male part of the flower and is composed of a filament as well as an anther containing pollen grains. Each floret carries one pistil that has a unilocular carpel (Murai et al., 2002) and can be developed into one caryopsis. Wheat caryopsis is often referred to as the seed in wheat literature.

Wheat is a staple crop and its seeds are the main part involved in harvesting. Wheat is the most important food in the world (www.idrc.ca/en/ev-31631-201-1-DO_TOPIC.html) and its significance increases along with the global population. Given the limited arable land, improving the wheat yield is essential (Peng et al., 2004). One way to improve the wheat yield potential in agriculture is to increase the grain number per spike (Dencic, 1994; Frederic and Bauer, 2000). For this purpose, a wide genetic variability in the wheat morphological structure has been considered by wheat scientists to obtain high grain numbers per spike (Sharman, 1944; Martinek, 1994). Three types of spike morphologies, namely, supernumerary spikelets, multi-spikelet (Peng et al., 1998), and multi-row spike (Martinek and Bednar, 2001), were considered as an ideal type for high yield wheat breeding because they can increase

the spikelet and seed number per spike (Dobrovolskaya et al., 2009). Peng (2003) selected a three-pistil (TP) mutant with normal spike morphology which produced three pistils per floret. Consequently, a floret is enabled to develop into seeds as many as three, and hence the seed number per spike increases. The TP trait is found to be controlled by a single dominant gene *Pis1* on chromosome 2D (Peng et al., 2004, 2008; Tiwari et al., 2011). Using molecular technologies, Yang et al. (2011a, b) identified seven differentially expressed genes in TP mutant, and indicated that the overexpression of the genes may be attributed to the TP trait.

In this study, in the offspring of a TP mutant crossing with Chinese Spring, a pistillody mutant plant was observed to develop into a new pure line. We tentatively designated this mutant as HTS-1. The HTS-1 plants stably exhibited novel phenotype such that all or parts of the stamen were transformed into pistils or pistil-like structures. As a result, the florets of HTS-1 plants had more pistils than normal and had the potential to increase the seed number per floret, which is relevant to high-yield wheat breeding. Wheat floret is also considered extremely stable, and very few mutants have been reported. Studies on floret mutants result in a rather superficial understanding of the determination of floral organ identity in wheat plants. Hence, the variation in wheat floret of the HTS-1 mutant is interesting. Thus, HTS-1 is a significant material for studying the floral development of wheat. This paper describes some characteristics of this mutation line including its floret morphology and inheritance.

Results

Morphological structure of mutant floret

Pistillody was serendipitously observed in the experimental field. During the study on the TP mutant line, we crossed a TP mutant with common wheat variety Chinese Spring, and the offspring was further backcrossed with Chinese Spring to develop an isogenic line with the TP trait. In the backcross offspring, a novel plant that showed 4–6 pistils or pistil-like structures along with 0–2 stamens in a floret was observed by chance. Given that these backcross descendants carried the *Pis1* gene from TP mutant, they should have shown three pistils per floret. The increased pistil number was equal to the decreased stamen number in the same floret in the new-found mutant plants, thus suggesting that some pistils were transformed from the stamen in the same floret. Accordingly, we further self-pollinated the mutant plant to develop a pure line that showed stable pistillody and named it as HTS-1. In view of the origin of HTS-1, it was deemed a sib-line of CSTP.

Considering that CSTP plants carried the *Pis1* gene, their floret consisted of one lemma, one palea, two lodicules, three stamens, and three pistils (Fig. 1-a). Among the three pistils in CSTP plant floret, one was a normal main pistil and the other two were increased subsidiary pistils that were determined by *Pis1* gene. A sib-line of CSTP, HTS-1 also carried the *Pis1* gene. However, HTS-1 plants exhibited different florets, i.e., some of their stamens had been turned into pistils or a combination of stamen and pistil (Figs. 1-b to 1-d). The latter is called a pistil-like structure for convenience. The morphology of pistillody stamens showed continuous variation from a few stigmatic hairs on an anterior anther to the complete transformation into pistil form (Figs. 2-b to 2-f). Slightly transformed stamens (Fig. 2-b) had normal filament with an anther-like structure bearing a tuft of “stigma hairs” at the top. The completely transformed stamens (Fig. 2-f) did not show any resemblance to stamens and appeared like normal pistil, and they can be identified only by their location in the floret. All the transformed elements occupied the same position in the flower as the stamens.

To observe the structure of pistillody stamens, they were longitudinally sectioned and examined for histological modifications. The completely transformed stamen (cTs) contained ovule-like structures instead of tapetum and pollen grains (Figs. 3a and 3b). Some cTs showed well-developed ovules, and this kind of pistillody stamens can be called as extra pistil. The extra pistils have fertility as they are seed setting. To date, we only observed four wrinkled seeds in a pistillody floret (Fig. 1e), whereas five or six seeds were expected but not observed possibly because of the narrow spaces in the floret. The partially transformed stamen (pTs) contained ovule-like structures and pistil form (Figs. 3c and 3d). The ovule of pTs was underdeveloped and sometimes contained deformed pollen grains (Fig. 3d).

Different pistillody expression and fertility of HTS-1 plant

The pistil-like structures of HTS-1 floret exhibited variable morphologies (Fig. 2) that represented the pistillody degree. Some HTS-1 florets had three pistillody stamens, whereas others only had one or two. Considerable variations in the number of pistillody stamen existed among individual spikelets of a given spike, and even among individual florets in the same

spikelet. However, some orderly patterns were noted in the field experiment. On the upper part of the spike, 25% florets had all their three stamens transformed into pistils or pistil-like structures. However, only 9% of florets on the lower and middle parts of the spike exhibited the same. In the same spikelet, different florets showed different pistillody degrees. The central floret often showed the maximum pistillody degree, regularly diminishing from central floret to bilateral ones.

The seed-setting rate of HTS-1 plants was often very low. Under the natural pollination condition, only 15.3% of bilateral florets of spikelets at the middle part of the spike developed into seeds. When the spikes were bagged, the seed-setting rate of bilateral florets of spikelets at the middle part of the spike was 12.2%. Using HTS-1 plants as female parents to cross with normal wheat material Chinese Spring, the seed-setting rate was reached about 30% by hand pollination, although 100% seed-setting rate was expected to normal wheat cross test.

The pollen activities of the pTs were low as only 5% pollen grains were dark blue after I-KI staining. In contrast, up to 95% pollen activities were detected in the small quantity of normal stamen in the pistillody plants, which was similar to that of normal wheat plants.

Pistillody occurs during early-stage stamen development

To determine the developmental point, at which the stamens transformed into pistil-like structures in the pistillody florets, we analyzed the early developmental stage of young spikes in CSTP (normal) and HTS-1 (pistillody) by SEM (Fig. 4). Floret development began in the basal positions of each spikelet and progressed toward the distal positions. Therefore, we can observe floret initiation by its growth stage order within a spikelet. In the normal spikelet of CSTP the stamen primordia were clearly distinguishable from the pistil primordia because of their tetralocular forms (Fig. 4a). In contrast, the stamen primordia of HTS-1 never attained a tetralocular form, but resembled the pistil primordia (Fig. 4b). This finding indicated that the homeotic transformation of stamens into pistil-like structures occurred during early-stage stamen development.

Inheritance analysis of pistillody

The pistillody trait of HTS-1 was stably expressed over three growing seasons since 2009. From the 1081 plants investigated in the field in 2010, none was found to be normal. Thus, the penetrance of pistillody was high (up to 100%). No normal plant was segregated from the HTS-1 growing population in recent years, indicating that it is already a pure line and can be used in inheritance studies. When the HTS-1 was crossed with the wild-type commercial wheat varieties, namely, Mianmai 45, Chuanmai 44, Chuanmai 28, and Chuanmai 50, all the F_1 hybrids showed wild-type phenotypes whether HTS-1 was used as the male or female parent. The pistillody trait is thus recessive. All the F_2 populations showed statistically typical Mendelian segregation of 3:1 or 15:1 (Table 1). The possibility of cytoplasmic influence on the pistillody of F_1 plants and F_2 segregation ratios was tested in a reciprocal cross of HTS-1 \times Chuanmai 28. Data (Table 1) indicated no cytoplasmic effect. The BC_1 segregation ratios in the cross between the pistillody line HTS-1 and the four commercial wheat varieties showed good fits to 1:1 and 3:1 ratios, respectively (Table 2). Thus, the pistillody trait is controlled by the interaction of two recessive karyogenes.

Table 1. Segregation for pistillody in F₂ populations derived from crossing HTS-1 to commercial wheat varieties

Combinations	Number of total plants	Number of normal plants	Number of mutant plants	Expected segregation ratio	χ^2	P
HTS-1/Mianmai 45	114	92	22	3:1	1.9760	0.20~0.10
HTS-1/Chuanmai 44	236	184	52	3:1	1.1073	0.30~0.20
HTS-1/Chuanmai 50	274	257	17	15:1	0.0010	>0.90
HTS-1/Chuanmai 28	269	252	17	15:1	0.0022	>0.90
Chuanmai 28/HTS-1	216	203	13	15:1	0.0197	0.90~0.80

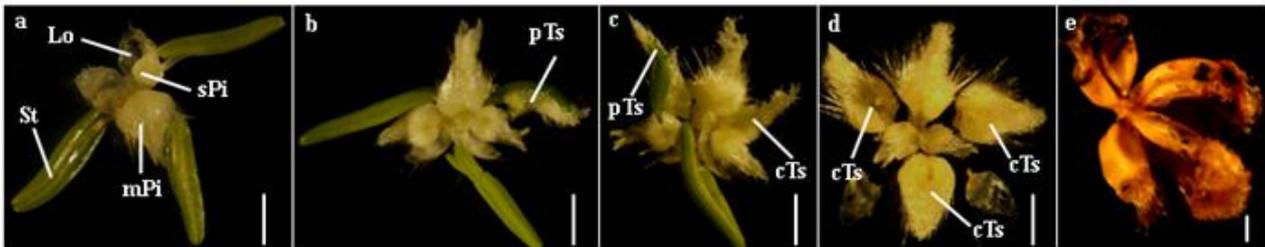


Fig 1. Morphology of the florets in HTS-1 and its sib-line CSTP plant. (a) A floret of CSTP plant; (b) one stamen had been turned into pistil-like structure in HTS-1 plant; (c) two stamens had been turned into pistil-like structure in HTS-1 plant; (d) three stamens had been turned into pistil-like structure in HTS-1 plant; (e) four wrinkled seeds in a floret of HTS-1 plant. St: stamen, mPi: main pistil, sPi: subsidiary pistil, Lo: lodicule, pTs: partially transformed stamen, cTs: completely transformed stamen. Scale bar represents 1mm.

The two recessive genes are temporarily designated as *hts1* and *hts2*.

Discussion

The differentiation of male and female sex organs in the sporophyte of angiosperms occurs very early in floral development, and is usually complete and irreversible. But it is also reported that there exist homeotic transformation of stamens into pistil-like structures in some species, such as in *Arabidopsis* (Got and meyerowitz, 1994; Jack et al., 1992), *Antirrhinum* (Sommer et al., 1990) and rice (Zhang et al., 2007). When the stamen of wheat flower showed various degrees of transformation towards the pistil form, the phenomenon is called pistillody (Leighity and Sando, 1924). Wheat pistillody plants were occasionally observed in interspecific hybrid populations (Kihara, 1951). Murai and Tsunewaki (1993) developed ulteriorly an alloplasmic line of wheat cv. Norin 26 (N26) with *Ae. crassa* cytoplasm and found that they have pistillody under long-day conditions of 15 h or longer. In the same way, Murai et al. (2002) reported another pistillody wheat material (cr)-CSdt7BS with *Ae. crassa* cytoplasm. In recent years, these two lines were used as research materials to probe into the genetic and molecular mechanism of wheat pistillody (Hama et al., 2004, Meguro et al., 2003, Mizumoto et al., 2009, Saraike et al., 2007, Yamada et al., 2009, Zhu et al., 2008). In the present study, a novel pistillody mutant was identified, which was named HTS-1. HTS-1 is genetically different from the pistillody wheat N26, and (cr)-CSdt7BS. The pistillody phenomena of N26 and (cr) CSdt7BS are caused by unclear-cytoplasm interaction (Murai et al., 2002, Zhu et al., 2008). However, reciprocal cross test indicated that the cytoplasmic effect on pistillody trait of HTS-1 does not exist (Table 1). In this study, the inheritance analysis showed that the segregation patterns of normal plants and pistillody plants in the cross progenies of HTS-1/Mianmai 45 and HTS-1/Chuanmai 44 could be explained by one-locus model, because 1:1 and 3:1 ratios exist in backcrossed and selfed progenies, respectively (Table 1, 2). Nevertheless, in the cross progenies of HTS-1/Chuanmai 50 and HTS-1/Chuanmai 28, 3:1 and 15:1 ratios exist in

backcrossed and selfed progenies, respectively (Table 1, 2), which matched with a two locus model. So, we concluded that pistillody trait of HTS-1 is determined by the interaction of two recessive karyogenes. We tentatively named them *hts1* and *hts2*. The special genetic base for pistillody trait of HTS-1 means it is a new kind of pistillody mutant.

Previous studies consider wheat pistillody as a character of low penetrance. Kihara (1951) as well as Kihara and Tsunewaki (1961) emphasized that environmental factors, particularly day length, are potentially important in the expression of the pistillody. The frequency of plants showing mutant phenotype is reported to vary from 3% to 18% in the progeny of self-pollinated pistillody plants in three seasons (Bhatia and Swaminathan, 1963). The low-penetrance characteristic seriously hampers intensive pistillody wheat studies. Fortunately, the new-found pistillody common wheat HTS-1 had high (up to 100%) penetrance under normal cultivation conditions. Field experiments revealed no normal plant in the HTS-1 population. The alloplasmic lines of wheat cv. Norin 26 and (cr) CSdt7BS with *A. crassa* cytoplasm had high penetrance under long-day conditions, but its pistillody cannot be observed under natural cultivation conditions (Murai and Tsunewaki, 1993, Murai et al., 2002). On the contrary, the pistillody of HTS-1 is not sensitive to photoperiodic change. It showed the same pistillody penetrance under both long- or short-day conditions (data not shown). The high-penetrance characteristic of HTS-1 under natural cultivation conditions proves its advantages in commercial wheat improvement and floral development studies.

Similar to a previous finding, the expression of pistillody was usually incomplete in HTS-1. In this study, most pistil-like structures in the HTS-1 floret were often not real pistils and variable in outward appearance as well as histological structures. However, the expressivity was variable in the filial generations of HTS-1 crossing with commercial wheat, and some plants showed higher expressivity than HTS-1. This finding suggested that some minor genes modified the expression of pistillody, thus enabling the development of complete expressive pistillody wheat (male sterile) lines by gene pyramiding.

Table 2. Segregation for pistillody in BC₁ populations derived from backcrossing HTS-1 to the F₁ hybrids of HTS-1 × commercial wheat varieties.

Combinations	Number of total plants	Number of normal plants	Number of mutant plants	Expected segregation ratio	χ^2	P
HTS-1// HTS-1/Mianmai 45	207	97	110	1:1	0.8164	0.50~0.30
HTS-1// HTS-1/Chuanmai 44	130	61	69	1:1	0.4924	0.50~0.30
HTS-1// HTS-1/Chuanmai 50	112	82	30	3:1	0.1905	0.70~0.50
HTS-1// HTS-1/Chuanmai 28	142	102	40	3:1	0.7605	0.50~0.30

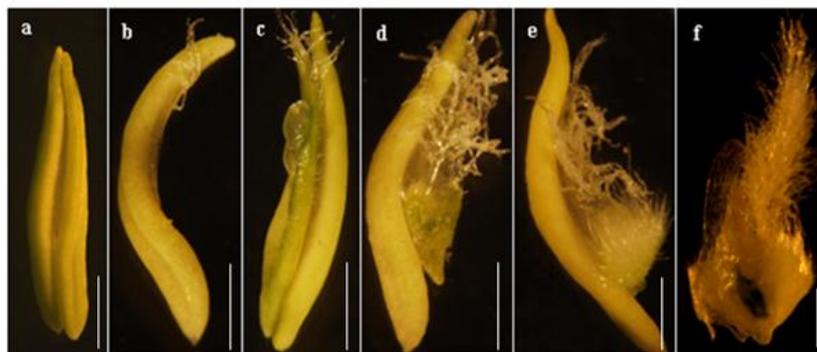


Fig 2. Normal and pistillody stamens in HTS-1 plant. (a) A normal stamen; (b) a few stigmatic hairs on anterior anther; (c) slightly transformed; (d-e) high degree of transformation; (f) stamen completely transformed into pistil. Scale bar represents 1mm.

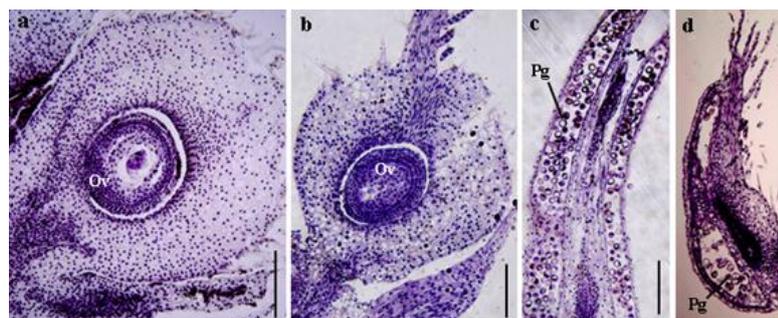


Fig3. Longitudinal sections of normal pistil and stamen in line CSTP and completely transformed stamen and partially transformed stamen in HTS-1. (a) a normal pistil in CSTP; (b) a completely transformed stamen in HTS-1 developing a normal ovule structure; (c) a normal stamen in CSTP; (d) a partially transformed stamen in HTS-1 contain ovule-like structures and pistil form. The ovule was underdevelopment and contains deformed pollen grains. Ov: ovule, Pg: pollen grain. Scale bar represents 0.5mm.

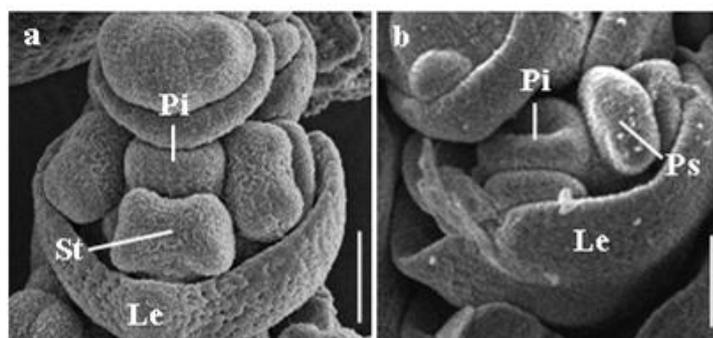


Fig. 4 SEM of young spikes in the lines CSTP (normal) and HTS-1(pistillody). (a) spikelet with developing florets in CSTP. Note that the developing stamen primordial begins assuming a tetralocular shape, which is distinguishable from the pistil primordial; (b) spikelet with developing florets in HTS-1. Note that the stamen and pistil primordial are distinguishable from each other in shape. Le: lemma, Pi: pistil primordial, St: stamen primordial, Ps: postillody stamen. Scale bar represents 200µm.

Materials and methods

Plant materials

HTS-1 is a new pistillody common wheat mutant found in our laboratory. Chinese Spring TP (CSTP), a near-isogenic line of common wheat variety Chinese Spring carrying the *Pis1* gene derived from the TP mutant (Yang et al., 2012) was used as a non-pistillody control in morphological analysis. HTS-1 was selected during the development process of CSTP. They are sib-lines that show similar phenotype except pistillody. Commercial common wheat varieties, namely, Mianmai 45, Chuanmai 44, Chuanmai 50, and Chuanmai 28 (provided by Dr. Wuyun Yang, Sichuan Academy of Agricultural Sciences) were used as references for the inheritance study of the pistillody trait.

Morphological analysis

For anatomical observation, the floral structures of the mutant and normal plant before flowering were observed under stereo microscope. The spikelet was investigated and some images were captured. For scanning electron microscopy (SEM) observations, young panicles and flowers were fixed following the method of Li et al. (2007). For light microscopic observations, wheat flowers were fixed in 50% ethanol, 0.9 mol/L glacial acetic acid, and 3.7% formaldehyde at 4 °C for 15 h. The specimens were stained with Alcian blue and dehydrated through a graded ethanol series, infiltrated with xylene, and then embedded in paraffin. Sections with 12 µm thickness were attached to gelatin-coated glass slides and observed under a light microscope.

Investigation of pistillody expression and fertility

The main-stem spikes of 100 plants were enveloped from the heading to the flowering stage. The numbers of normal and transformed stamens in the spikelet of the lower, middle, and upper parts of the spikes were recorded at the flowering stage. The total floret number and seed-setting number were recorded. Pollen was examined under microscopy. One anther was obtained from each spikelet, squashed with a glass rod on a glass slide to disperse pollen grains, and stained with 2% iodine-potassium iodine solution (I-KI). Only the round, normal-sized, and dark-blue stained anthers were considered fertile.

Inheritance analysis

The pistillody mutant HTS-1 was crossed with Mianmai 45, Chuanmai 44, Chuanmai 50, and Chuanmai 28. Some F₁ hybrids were self-pollinated to obtain F₂ seeds, and the others were backcrossed with HTS-1 to obtain BC₁ seeds. During the 2010–2011 growing season, the seeds of parents and offsprings (F₁, F₂, and BC₁) were sown in the same experimental field on the same day. All seeds of each population were randomly planted with labels for identification. The numbers of pistils and stamens in the florets of each plant were recorded during the flowering period. Plants were classified as pistillody if any anther displayed pistillody characteristics.

Statistical analysis

A chi-square (χ^2) goodness of fit test was performed on the test cross progenies against a possible theoretical segregation ratio using the formula: $\chi^2 = \sum (O-E)^2/E$, where, O and E are the observed and expected values, respectively (Steel and Torrie,

1980).

Conclusion

HTS-1 stably showed pistillody under normal cultivation conditions. One to three stamens of its floret were turned into pistils or pistil-like structures. The homeotic transformation occurred during early-stage stamen development. HTS-1 is genetically different from the pistillody wheat lines (cr)-CSdt7BS and N26 reported before. Inheritance analysis indicated that the pistillody trait of HTS-1 is determined by the interaction of two recessive karyogenes, *hts1* and *hts2*. The special genetic base for pistillody trait of HTS-1 means it is a new type of pistillody mutant. HTS-1 showed high penetrance, which proves its advantages in commercial wheat improvement and floral development studies.

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