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Morphological, Physiological and Molecular Markers for the Adaptation of Wheat in Drought Condition

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Author's contribution

The sole author designed, analysed, interpreted and prepared the manuscript.

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Review Article

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ABSTRACT

Globally wheat is most important crop and mostly grows in rainfed areas. In cereal crops, wheat having highest protein content. In the abiotic stresses, mostly drought effects wheat productivity and at growth stages. According to climate change, frequency of drought increases in arid and semi-arid region because of water shortage. Drought effects all growth stages of wheat and more critical at flowering and grain filling stage. Losses of wheat productivity depend on the severity and duration of drought because of reducing in photosynthesis, stomata closure, metabolic activity decrease, oxidative stress increase and result in poor grain formation ultimately yield loss. Easy method to get yield from drought areas are to develop drought tolerance genotypes according to marks. Heritable variation required for the improvement, but heritability is low because of the genotypic and environmental interaction. Different genotypes of wheat behave different in drought. A comprehensive study helps us understanding of some important markers. Breeders can select well adaptive drought genotypes on the base of morphological markers (avoid leaf senescence, flag leaf, root system, grain development, stay green character, cuticular wax and stomata conductance.), physiological markers (abscisic acid (ABA), proline, chlorophyll content, jasmonic acid (JA) and cell stability) and molecular markers (Dreb 1, Dreb 2, Rht 8, TaMYB33, TaRZF38 etc.). Several genes which are doing job for drought stress tolerance and change the enzymes and proteins like, late embryogenesis abundant, rubisco, responsive to abscisic acid, glutathione-S-transferase, carbohydrates, helicase, and proline during drought stress. Drought stress alters some gene

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expression and cannot work properly due to the influence of environmental factors. Researchers used biotechnological tools to identify the specific genes for drought tolerances. These markers help us to identified drought tolerance genotypes for breeding program. This review paper covers morphological, physiological and molecular marks for the development of drought tolerance genotypes.

Keywords: Wheat; drought; tolerance; morphological; physiological; molecular; markers.

1. INTRODUCTION

In cereal crops, wheat (*Triticum aestivum*, 2n=6x, AABBDD) is staple food of more than 35% world population [1]. Increase in population, bread wheat becomes more significant for human [2]. Drought rapidly increases in wheat producing rainfed areas like southern Australia, Africa, and Mediterranean region [3]. Wheat is important source of protein for human and having high protein content as compare to rice, maize and other cereal crops. Wheat has more than half calories and almost half protein [4].

According to climate change, frequency of meteorological events and causes gain the attention of the world. The gap between food production and demand are due to abiotic stress like drought, high temperature, frost, etc. [5]. One of most important abiotic stress is a drought which cause by low rainfall and also effects agricultural production. It defines as lack of moisture in the soil which does not fulfill the requirement of plant and disturb plant from normal activities [6]. National science foundation (NSF) reported that drought will be more in next 30 years and badly effect the crop yield by 6-12 bushel/acre [7]. Periodic drought effects more than 50% of the area under wheat cultivation [8]. Drought stress reduces water potential of the cell, turgor pressure, growth of plant and their biomass. It is particular to occur in arid and semi-arid areas. Drought mainly effects the rate of photosynthesis, cell division, elongation, root proliferation, disturb water and nutrients relationship [9]. Reactive oxygen species (ROS) produce as result of drought which effect the cellular mechanism, enzyme inhibition, protein degradation, effect on DNA and RNA at the end cell death [1]. Drought also effects the reproductive organs, grain filling stage, pollen viability and seed development [10]. In recent years, agricultural management practices like irrigation and crop improvements play important role in increasing grain yield [6]. Short duration varieties develop for predictable rainfall areas. In unpredictable rainfall environments transpiration water left in soil at the time of maturity and yield sacrificed [11]. For plant breeders, abiotic stress tolerance is a big challenge because of high genotype x environment interaction, low heritability, and mutagenic nature of abiotic stress responses [12].

Majority of breeding program has principal goal for improvement of drought tolerance for a long time [13]. Improvement occurs by empirical breeding, in which yield was taken as a main marker for selection in target environment [14]. Morphological, physiological and biochemical markers offer for consideration as selection criteria for screening drought tolerance in wheat [15]. In wheat, major limiting factor is narrow genetic variation in D-genome. Synthetic hexaploid wheat (SHW) was produced artificially to increase diversity in D-genome for drought tolerance [2]. This review covers the most beneficial morphological, physiological and molecular markers, breeder can be used for drought tolerance in wheat crop.

2. MORPHOLOGICAL MARKERS

Various morphological markers use for screening of drought tolerance genotypes. Wheat genotypes shows positive correlation in leaf area, height and yield [16]. Drought tolerance genotypes produce more leaf area and total dry matter in drought stress [17]. Breeder use morphological markers i.e. leaf senescence [8], flag leaf [18], stomata conductance [19], grain development [20] and root system [21] as selection criteria in drought tolerance genotypes.

2.1 Leaf Senescence

Leaf senescence is defined as change in leaf color due to chlorophyll and membrane breakdown. It effects the functions of leaf because of water decrease with age [8]. Chlorosis is primary sign of leaf senescence. It is due to decrease in photosynthesis [22]. Drought during grain filling stage reduces the grain filling period [23]. Early leaf senescence occurs due to continuous water deficit condition [24]. Flag leaf assimilates (30-50% of total) during grain development in wheat. Leaf senescence increase according to drought increase, drought stress occurs at reproduction stage cause in reduce grain yield [8]. Wheat genotypes produce better yield that sustain leaf photosynthesis for longer time [25].

2.2 Flag Leaf

In morphological markers, flag leaf effect plant architecture and yield potential in wheat. In favorable condition flag leaf of some wheat genotypes contributes 45–58% in photosynthesis activity and after flowering 41-43% use in grain filling [18]. During the reproductive stage, flag leaf provides assimilates for plant growth, development, spike development, drought adaptation signal and photosynthesis [26]. Flag leaf characteristics i.e. size, width, length, flag leaf angle [27] are positively correlated with the yield in cereal crops [28]. Wheat genotypes with smaller and more erect flag leaves are able to reduce water loss due to rolling their leaf in drought and give high yield as compare to lax leaf genotypes [18]. Characteristics and function of flag leaf are closely related to grain filling in wheat [19].

2.3 Stomata Conductance

In the initial of drought, stomatal conductance reduced because of reduced photosynthesis. In some conditions, non-stomatal and metabolic inactivity cause increase in CO_2 and close temporary stomata [8]. Drought at later stages cause dehydration in tissue and effects the metabolic activity [29]. Water loss effects the photosynthesis, reduced turgor, stomatal conductance, reduce growth, leaf water potential and reduce yield. Stomata conductance which can contribute to continued growth under water stress use as identification in drought tolerance. [30]. Leaf epidermal cells stomata uptake CO_2 in photosynthesis and water loss with transpiration. Mechanism of stomata opening and closing can reduce the water loss and high photosynthetic rate maintain. Stomata density and size determinates of water loss and growth [19].

2.4 Grain Development

In cereals grain development initiate with the fertilization of egg to form zygote and one nuclei form endosperm [31]. Photosynthesis occur in leaves and store food in vegetative parts that play important role in grain filling [20]. At young microspore stage of pollen, drought creates sterility in pollen and reduces in grain number [32]. In drought meiosis and anthesis are badly affected at the end reduce grain yield [33]. Grain number in wheat shows no effect of drought and has effect on grain filling result in shorten the grain filling stage [8].

2.5 Root System

For yield improvement, plant root systems gain attention as morphological marker [21]. From many years, breeding for high yield with high input create narrow genetic germplasm and with loss of well adaptive markers [34]. Root markers are polygenic in nature that effect root function and architecture [35]. Breeder continuous work on identification of root makers that make plant to adapt drought environment. Hydraulic conductivity increases because of more root depth and crop take water from depth at grain filling stage [36], root length density, small root diameter, and large specific root length play role in drought tolerance [21] and angle between seminal roots should be optimized [37].

2.6 Stay Green Character

It is defined as the ability of plant to remain photosynthetically active due to delayed senescence is called stay green character. Its duration in flag leaf and harvesting index is positively correlated with water use efficiency during grain development. The genotypes which sustain flag leaf photosynthesis produce more yield as 30-50% photosynthates require during grain filling [8]. [38] concluded correlation analysis of different genetic traits in wheat with grain yield and found highly significant correlation between flag leaf area persistence and maintain yield in droughts. [39] also concluded positive correlation of green flag leaf with wheat yield. Increase in grain filling and improvement of desirable traits, at the end increase in grain yield [40].

2.7 Cuticular Wax

Wheat leaves with glaucous characteristics are coated with wax. Six genes controlling wax have been reported and located on wheat chromosomes W1 and IW1 on 2BS, W2 and IW2 on 2DS, W3 on 2BS, and IW3 on 1BS [41,42]. Leaf cuticular wax can protect the plants against abiotic and biotic stresses [43]. Firstly, cuticular wax observed in drought stress in plants, such as tobacco, alfalfa, rice and wheat that play role in drought tolerance and leaf water potential decreased under drought tolerance, which is essential to keep plants having relatively high photosynthesis rate and relative high yield. Leaf cuticular wax on wheat drought tolerance in an attempt to develop drought resistance cultivar [44].

3. PHYSIOLOGICAL MARKERS

In susceptible wheat genotypes yield contributing markers and yield reduction is observed. In physiological markers and yield have positive relationship. Physiological marks help in understanding plant growth and product in drought stress [45,46]. Drought tolerance genotypes can be developed by using physiological markers as selection criteria. Researchers fined physiological markers i.e. high chlorophyll content [16], high proline content [47], cell membrane stability [48] and jasmonic acid [49] that make plant mechanism to tolerant drought stress [16].

3.1 Abscisic Acid (ABA)

In drought condition, Abscisic acid (ABA) increases in plant. Drought tolerance genotypes produce abscisic acid (ABA) that help in adaptation of drought condition. ABA hormone produce in many stresses and responses by modification of protein synthesis [50,51,52,53]. Increase in relative water content due to increase in ABA in drought condition [54]. Lower ABA concentration in reproductive organs result in higher grain yield and sign of drought tolerance. An Ideal genotype has optimum root depth, water transportation, low ABA and high stomatal sensitivity [52]. Increase in activities of antioxidant enzymes [55] e.g. peroxidase (POD) and superoxide dismutase (SOD) due to increase in ABA [56]. In drought condition, ABA increase in flag leaf and also increase grain yield. At booting stage, ABA in flag leaf significantly increase and at anthesis stage ABA prominently decrease [24].

3.2 Proline

Proline is a protein which produce in plants under stress environment. Proline function in stress environment e.g. redox potential in the cell, destroying free radicals, osmotic adjustment and stabilizing sub cellular structures. Proline does not disturb the normal cell biochemical reactions and support plant to survive in stress. In water stress and salinity, proline concentration increases in plant parts [47]. Proline produces in plant body by glutamic acid pathway [57]. In drought, wheat plant response rapidly and produce more proline amount as compare to other osmoregulators. Beneficial organic solutes produce that prevent water loss. Proline also protects the cell from ultraviolet radiation. It helps us to understand the mechanism of drought tolerance in wheat. It more produces in drought condition to help plant to survive [58]. Proline accumulation and drought tolerance shows positive correlation with each other. Different wheat genotypes have their own threshold level in drought condition. Proline accumulation in different wheat genotypes use as marker in drought tolerant plant [59].

3.3 Chlorophyll Content

In drought condition chlorophyll content decrease [60] and chlorophyll b reduce more as compare to chlorophyll a. Drought tolerance genotypes have high chlorophyll content in drought stress [61]. Chlorophyll content uses as marker for evaluation of germplasm. In drought chlorophyll decrease and stomata's effect. Chlorophyll and higher carotenoids associated with chlorophyll fluorescent in drought tolerance. Chlorophyll efficiency of a plant with 4 carbons at temperature 30 to 45°C and plants with 3 carbons at a temperature of 10 to 25°C has best chlorophyll yield. When the leaf emergence until its full growth, increases in photosynthetic growth rate and then decrease gradually [62]. Active oxygen species effect the chloroplast and result in decrease in chlorophyll. Severe drought stops the activity of photosynthesis at the end effect the chlorophyll component, chlorophyll content and photosynthetic apparatus [63]. Photo-synthetic capacity is positively correlated with leaf chlorophyll. Drought sensitive genotypes rapidly decrease chlorophyll content. Tolerant genotypes with high chlorophyll content considered as a favorable marker. Chlorophyll content use as physiological marker for drought tolerance in wheat [16].

3.4 Jasmonic Acid (JA)

Plants show different physiological and structural modification in an environment [64]. JA helps in the germination of dormant seeds [65]. JA founds abundantly in the chloroplast [66]. Plant produce variable and non-variable compounds that consist on phytohormones to adapt a changing environment [67]. Most important hormones and jasmonic acids (JA) its methyl ester methyl jasmonates (MeJAs), drive from fatty acid ([68]. JA is commonly found in plant kingdom [49]. Firstly, fungus Lasiodiplodia theobromae used for isolation of JA [69]. JA plays role in developmental, physiological activities, growth, oxidative defense, reproductive processes, root elongation, fruit ripening, sex determination, fertility, biotic and abiotic stress tolerance [70]. It involves in regulation of tolerance against different environmental stresses [71].

3.5 Cell Membrane Stability (CMS)

In drought tolerance in wheat genotypes, conductivity test and mitochondrial cell viability use to measure cell membrane stability (CMS) and reduction in tetrazoliumtriphenyl chloride test (TTC) take considerable attention [48]. Cell membrane disruption due to crowding of the cellular components that may be due to decrease cellular volume and at the end protein denatures [72]. CMS use as indicators of drought tolerance and cell membrane injury measure by electrolyte. Drought tolerant genotypes show high cell membrane stability then the susceptible genotypes [16]. Cell membrane stability and grain yield show the positive correlation in stress condition. CMS measurement uses as selection for drought tolerant genotypes [59].

4. MOLECULAR MARKS AND QUANTITATIVE TRAIT LOCI (QTLs)

QTLs analysis through molecular makers showed that chromosome 5B, 4B and 7B having important genes for drought tolerance in wheat. QTLs discovered on chromosome 5B placed between two markers (M51P65 and Psr136) have positive correlation with drought tolerance. QTLs on chromosome 4B and 7B placed between markers (M62P64d- Rht and M83P65d - M21P76n) have negative effect on drought tolerance [73]. On chromosome 4A have marker (Xwmc89) showed important relationship with drought tolerance [74].

Best method for the development of drought tolerance is molecular mapping and marker assisted selection. In some wheat genotypes, amplified fragment length polymorphism (AFLPs), restriction fragment length polymorphism (RFLPs), microsatellites (SSRs), SNPs, RAPDs and simple sequences repeat (SSR) markers used for mapping of senescence of flag leaf shown in Table 1. QTLs mapping detected gene on chromosome 2D having better performance in drought [75]. Milad in 2011 [76] Identified RAPD and ISSR makers associated with senescence of flag leaf in drought.

Molecular markers are best techniques for breeder. RAPD shows rapidly result but limitation is low reproducibility. In their opposite ISSR markers are more reproducible and highly informative. ISSR markers used in cereals for genetic diversity, gene mapping, phylogenetic relationship and DNA finger printing [77].

Table 1. Primers name and sequences of RAPD, ISSR and microsatellite primers used for PCR

Markers	Primer names	Primer sequences (5' to 3')	References
RAPD	OPE-26	5' AACGGTGACC 3'	[77]
	A-12	5' TCGGCGATAG 3'	
	E-10	5' CACCAGGTGA 3'	
	OPT-08	5' AACGGCGACA 3'	
	OPC-19	5'GTTGCCAGCC 3'	
	OPX-17	5' GACACGGACC 3'	
	A-02	5'TGCCGAGCTG 3'	[78]
	A-03	5'AGGGGTCTTG3'	
	A-04	5'AATCGGGCTG3'	
	A-08	5'GTGACGTAGG3'	
	A-10	5'GTGATCGCAG3'	
	A-13	5'CAGCACCCAC3'	
	A-15	5'TTCCGAACCC3'	
	A-16	5'AGCCAGCGAA3'	
	A-17	5'GACCGCTTGT3'	
	A-20	5'GTTGCGATCC3'	
	B-05	5'TGCGCCCTTC3'	
	B-07	5'GGTGACGCAG3'	
	B-10	5'CTGCTGGGAC3'	
	B-17	5'AGGGAACGAG3'	
	B-19	5'ACCCCCGAAG3'	

Markers	Primer names	Primer sequences (5' to 3')	References
	Pr_1	5'CAGGCCCTTC3'	[79]
	Pr_3	5'AGTCAGCCAC3'	
	Pr_4	5'AATCGGGCTG3'	
	Pr_5	5'AGGGGTCTTG3'	
	Pr_6	5'GGTCCCTGAC3'	
	Pr_7	5'GAAACGGGTG3'	
	Pr_8	5'GTGACGTAGG3'	
	Pr_9	5'GGGTAACGCC3'	
	Pr_{10}	5'GTGATCGCAG3'	
	Pr_{11}	5'CAATCGCCGT3'	
	Pr_{12}	5'TCGGCGATAG3'	
	Pr_{13}	5'CAGCACCCAC3'	
	Pr_{14}	5'TCTGTGCTGG3'	
	Pr_{15}	5'TTCCGAACCC3'	
	Pr_{16}	5'AGCCAGCGAA3'	
	Pr_{17}	5'GACCGCTTGT3'	
	Pr_{19}	5'CAAACGTCGG3'	
	Pr_{20}	5'GTTGCGATCC3'	
	OPA02	5'TGCCGAGCTG3'	
	OPA07	5'GAAACGGGTG3'	
	OPB09	5'TGGGGGACTC3'	
	OPB13	5'TTCCCCCGCT3'	
	OPC04	5'CCGCATCTAC3'	
	OPC15	5'GACGGATCAG3'	
	OPE20	5'AACGGTGACC3'	
	OPF15	5'CCAGTACTCC3'	
ISSR	M-1	5' (AC) 8 CG 3'	[77]
	UBC-811	5' (GA) 8 C 3'	
	UBC-817	5' (CA) 8 A 3'	
	UBC 814-32	5'(CT) 7CCTA 3'	
	AD1	5'(GA)9C3'	[79]
	AD2	5'(AGC)6G3'	
	AD3	5'(ACC)6G3'	
	AD5	5'(CA)10C3'	
	AD6	5'GT(CAC)73'	
	AD9	5'(AC)9G3'	
	M-1	5'(AC)8CG3'	
	M-6	5'(CAC)53'	
	M-7	5'(CAG)53'	
	M-8	5'(GTG)53'	
	SSR-1	5'(GA)8T3'	
	ISSR-3	5'(CT)8A3'	
	ISSR-4	5'(CT)8G3'	
	ISSR-5	5'(TC)8A3'	
	ISSR-808	5'A(GA)7GC3'	
	ISSR-811	5'G(AG)7AC3'	
	ISSR-816	5'C(AC)7AT3'	
Microsatellite	Xgwm 186	5'GCAGAGCCTGGTTCAAAAAG3'	[80]
	Xgwm 337	5'CCTCTTCCTCCCTCACTTAGC3'	[81]
	Xwmc 89	5'ATGTCCACGTGCTAGGGAGGTA3'	[82]
	Xgwm 108	5'CGACAATGGGGTCTTAGCAT3'	[83]

Abiotic stress induced through transcription elements i.e. Dehydration responsive element binding (DREB). Dreb 1 genes are placed on 3A, 3B and 3D chromosomes in wheat. Due to mapping, Dreb B1 gene placed between Xmwg818 and Xfbb117 on the 3BL chromosome. Dreb B1 gene is responsible for drought, salinity, heat tolerance in wheat. Dreb1/Dreb2 genes isolated from *Triticum aestivum*, *Oryza sativa*, *Zea mays* and perennial ryegrass [84]. Wheat gene TaMYB33, detoxified reactive oxygen species (ROS), tolerance against salt and drought stresses [85]. Another wheat geneTaMYB2 conferred drought tolerance [86]. Wheat expansis protein (EXPB) play important role in cell wall extension during growth. Expression of TaEXPB23 gene response to water stresses [87]. Reduced height genes (Rht)

Table 2. Genes play vital role in drought tolerance in wheat

Sr. no.	Genes	References
1	Dreb 1	[86]
2	Dreb 2	[86]
3	Rht 8	[88]
4	TaMYB33	[85]
5	TaRZF38	[90]
6	TaRZF70	[90]
7	TaRZF74	[90]
8	TaRZF59	[90]
9	TaVP3	[56]
10	TaVP2	[56]
11	TaVP1	[56]
12	TaEXPB23	[87]
13	TaMYB2	[86]
14	TaNAC2a	[92]
15	TaMYB30-B	[42]
16	R2R3- MYB	[42]
17	TaWRKY19	[42]
18	TaWRKY2	[93]
19	TaSIP	[93]
20	TaSRHP	[93]
21	TaHPS	[93]
22	TaASR1	[93]
23	TaNAC2a	[94]
24	TaNAC13	[94]
25	TaNTL	[94]
26	TaNAC7	[94]
27	TaNAC4a	[94]
28	TaNAC6	[94]
29	TaWRKY10	[95]
30	TaWRKY1	[95]
31	TaWRKY33	[95]
32	TaWRKY93	[95]
33	TaWRKY44	[95]
34	TaRAP2.1	[95]
35	TAZFP34	[95]
36	TaERF1	[95]
37	TaERF3	[95]
38	Xcfd22-7B	[96]
39	Xcfa2114-6A	[96]
40	Xgwm181-3B	[96]
41	Xwmc405-7B	[96]
42	Xgwm148-3B	[96]
43	Xwmc166-7B	[96]
44	TaSnRK2.7-a	[42]
45	TaSnRK2.7-b	[42]
46	TaSnRK2.7-c	[42]
47	TaSnRK2.7-d	[42]
48	TaSnRK2.7-e	[42]

in wheat that makes short stature. Dwarfing genes Rht- B1b, Rht-D1b and Rht8 are identified and have positive correlated with drought tolerance in wheat [88].

Vacuolar H+translocating pyrophosphatase (V-PPase) is an enzyme that have an important role in development of plant and tolerant to abiotic resistant and wheat V-PPase genes, TaVP3, TaVP2, and TaVP1 play role in drought tolerance [89]. [87] develop a transgenic tobacco having gene TaEXPB23 that showed water retention ability (WRA). TaEXPB23 gene may be used in wheat genotypes to develope water retention ability (WRA) for drought tolerance in wheat. Kam in 2007 [90] discovered responsible genes. TaRZF38 and TaRZF70 RING-H2 that up regulated in leaf and down regulated in roots, TaRZF74 and TaRZF59 were expressed in embryo and endosperm at the highest level in wheat during water stress. Myeloblastosis oncogenes (MYB) play important role in growth, development and response to stress. TaMYB30 and TaMYB30-B genes discovered that encoded for R2R3-type MYB protein [91]. Sucrose non-fermenting protein kinases 2 (SnRK2) show signaling in stress plant. TaSnRK2.8 is a regulatory factor providing strength to plasma membrane stability. Drought, salt, cold tolerance produces in transgenic Arabidopsis due to Overexpression of TaSnRK2.8 shown in Table 2.

5. CONCLUSION

In the abiotic stresses, drought is major environment stress that effects the wheat productivity worldwide. Crop stage, intensity and duration of drought determine the effect on grain yield. Drought effects the wheat plant during any stage of wheat but most devastating during reproductive and grain filling stage. Significant variation occurs in the wheat genotypes for drought. Drought stress can be minimized by drought tolerant genotypes and evaluating through morphological, physiological and molecular marks. Our knowledge of drought tolerance mechanism has been enhanced and focus on the morphological, physiological and molecular markers use for drought tolerance and it have significant effect on yield. Breeder can evaluate germplasm by using morphological, physiological and molecular markers for drought tolerance. Researchers trying physiological and molecular marks for improvement in genotypes against drought. To search allelic location for drought resistant and their introgression into high yield genotypes through mendelian genetics and present day biotechnological methodologies may enhance the tolerance against drought. New development techniques in sequencing, marker development, and genome analysis give the opportunity to identify the specific drought tolerances gene in genome. Morphological and physiological markers are cheapest and rapidly evaluate drought tolerance response then molecular markers.

COMPETING INTERESTS

Author has declared that no competing interests exist.

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